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THE UNIVERSITY OF ALBERTA

ASPECTS OF THE BIOLOGY OF AVIAN *SARCOCYSTIS*

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Aspects of the biology of avian *Sarcocystis*," submitted by Theodore Emile Drouin in partial fulfilment of the requirements for the degree of Master of Science.

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Date October 21, 1976

ABSTRACT

An investigation into selected aspects of the biology of avian *Sarcocystis* was conducted from May, 1973 to August, 1975. A total of 900 birds was examined grossly and 885 of those histologically for the presence of the parasite. Different prevalences were obtained for several species, and nine new host records for *Sarcocystis* in birds were reported.

The prevalence of *Sarcocystis* in some anatids in Alberta was found to be lower than that reported in the same anatid species in two other studies. Different migratory routes and different overwintering grounds for the ducks in each of the three studies are suggested as explanations for these findings. This hypothesis is supported by statistical analysis of the data for shovelers (*Anas clypeata*) and by failure to complete the life cycle of the macrocyst-producing sarcosporidian of shovelers in mammals commonly found in the Temperate Zone.

Histological examination of muscle samples from all the birds for the presence of microcysts showed that prevalence data based exclusively on the presence of macrocysts are underestimations of actual prevalences of infection in some hosts. Gross examination by itself may indicate that some species are uninfected, when in fact they are frequently infected.

An intensive study of the cyst wall was undertaken with the light microscope. Two fundamentally different types of cyst wall were found; they were characterized by the presence, or absence, of external villosities. Within these two types were distinguished seven sub-types.

Differences in cyst structure did not depend upon the host species infected.

Attempts to elucidate the life cycle of the macrocyst-producing parasite of ducks were unsuccessful but preliminary results with a microcyst-producing species in black-billed magpies (*Pica pica*) indicated a domestic cat-bird cycle with the production of isosporan oocysts which resembled those of *Isospora felis*. Confirmation of the cycle by establishment of sarcosysts in pintails (*Anas acuta*) and Japanese quail (*Coturnix coturnix*) was unsuccessful.

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1. INTRODUCTION

Parasites belonging to the genus *Sarcocystis* represent a ubiquitous group: animals from fish to mammals have been reported infected with its cystic stages. The recent discovery of the life cycle of *Toxoplasma gondii* (Hutchison, 1965, 1967; Work and Hutchison, 1969; Frenkel *et al.*, 1970) provided the clue which led to the discovery of the life cycle of *Sarcocystis*. Fayer (1970) demonstrated the formation in tissue culture of gametocytes and oocysts by endozoites of an unnamed species of *Sarcocystis* taken from intramuscular cysts from grackles. Rommel *et al.* (1972) demonstrated isosporan-like oocysts and sporocysts in the feces of cats fed cysts of *S. "tenella"* from sheep. Dogs and cats fed beef infected with *S. "fusiformis"* passed isosporan sporocysts (Heydorn and Rommel, 1972). This was confirmed by Mahrt (1973), Fayer *et al.* (1973), Fayer and Johnson (1974), and Munday *et al.* (1975). Thus the coccidian nature of *Sarcocystis* is now well established, and the route of infection seems to be oral. Carnivores become infected with enteric stages when they eat sarcocyst-infected meat, and herbivores become infected with the muscular stages when the oocysts or sporocysts from carnivore feces are ingested.

As a result of these findings it has been shown that *S. "fusiformis"*, which until now referred without qualification to any *Sarcocystis* found in beef, constitutes a species "complex" with a minimum of at least three valid species (Mehlhorn *et al.*, 1975a, b; Heydorn *et al.*, 1975a, b; Gestrich *et al.*, 1975). Criteria which were used for identification of the individual species include life cycle, morphology of cyst wall, size

and arrangement within the cyst of the endozoites (merozoites of some workers), and developmental sequences within the herbivore host (hereafter referred to as the intermediate host) (Heydorn *et al.*, 1975b). Similarly, *S. "tenella"*, characteristically found in sheep, has been shown to constitute at least two species. One species uses cats as the carnivore host (hereafter referred to as the definitive host) (Heydorn and Rommel, 1972), and the other uses canids as the definitive host (Ford, 1974; Munday *et al.*, 1975). The taxonomy of the species complex of *S. "fusiformis"* was discussed and amended by Heydorn *et al.* (1975b).

Scott (1943a, b) reviewed the early literature on mammalian *Sarcocystis*, especially *S. "tenella"*. In these reviews he cited many early workers who presented evidence of the pathogenic effects of the parasite in the intramuscular stages. This evidence dealt with the response of the host when the sarcocysts became senescent. More recently, Fayer and Johnson (1973, 1974), Fayer *et al.* (1973), and Johnson *et al.* (1975) reported pre-cystic pathology and death of calves fed sporocysts from dog feces.

Despite the intensive research on the mammalian sarcosporidians, to date little work has been done with this parasite in hosts from other classes. There is only one report with questionable results of a rat-snake host system (Rzepczyk, 1974), and two unsuccessful attempts to complete a life cycle with avian forms (Borts and Zwart, 1974; Shaffer, 1974).

Sarcocystis "rileyi" Stiles, 1893 was named for a macrocyst-producing sarcosporidian found in the pectoralis muscles of a mallard. It has been common practice to assign to this species any sarcosporidian

in ducks forming such macrocysts, and often the name *S. "rileyi"* has been assigned to any avian sarcosporidian, regardless of size of the cyst or host infected (Salt, 1958; Cornwell, 1963). Conversely, some workers have named new species solely on the basis of the host species infected (Crawley, 1914). Many refer to *Sarcocystis* sp. when discussing the presence of sarcosporidiosis in the avian host. A more detailed review of the literature on *Sarcocystis* is given in Appendix 3.

The literature on avian *Sarcocystis* was reviewed by Erickson (1940) and updated by Cornwell (1963). Both reviews are essentially checklists of hosts infected with species of *Sarcocystis*. Most reports are based on the fortuitous finding of macrocysts while some other aspect of avian biology was being investigated. Since the investigators did not look for microcysts, reliable estimates of prevalence of this parasite in birds are few. The studies of Selander (1955), Chabreck (1965), Fayer and Kocan (1971), and Hoppe (1976) are exceptions to the occasional nature of this research.

A further result of the occasional nature of most avian *Sarcocystis* studies is the absence of detailed studies of the cyst and its structures. The only modern exceptions are the ultrastructure studies of Zeve *et al.* (1966) and Simpson and Forrester (1973). Both these groups worked with a sarcosporidian found in grackles, but neither provided much enlightenment on the biology of avian *Sarcocystis*.

In view of the new information on the biology of the mammalian species of *Sarcocystis* and the paucity of information on the avian parasites of this genus, this study attempted to answer the following questions:

1. What is the prevalence of *Sarcocystis* in different bird species?
2. Is examination of birds for the presence of macrocysts a reliable indication of the prevalence of infection?
3. What is the life cycle of avian *Sarcocystis*?
4. Are there differences in the structure of the microcysts?
5. Is *Sarcocystis rileyi* a valid species, or is there more than one species of *Sarcocystis* in birds?
6. Is there any pathology associated with infection, and if so, at what stage does it occur?

2. MATERIALS AND METHODS

Samples of striated muscle were obtained from a number of sources including: birds collected on scientific permits held by various researchers, birds given to the University of Alberta by the Fish and Wildlife Division of the Alberta Department of Parks, Recreation, and Wildlife, birds from hunters' bags and from birds collected for this study. Many birds, especially those obtained from the Fish and Wildlife Division, had been dead and frozen for an undetermined period prior to this study. Most samples came from the Edmonton region of Alberta, but the blue grouse samples came from Vancouver Island, British Columbia.

All birds were identified to species using the Birds of Alberta (Salt and Wilk, 1966), and A Field Guide to Western Birds (Peterson, 1961) whenever an identity was uncertain. Scientific and common names are given according to the checklist of the American Ornithological Union (AOU) (1957) and an AOU supplement (1973). Common names only of birds will be used in the text; the corresponding scientific names are given in Appendix 1. Similarly, common names of mammals are used in the text and scientific names appear in Appendix 2.

When the entire carcass was available, a section of pectoralis muscle about one to two square centimeters in area was removed in a line between the humerus and about a quarter of the distance back along the keel of the sternum. Hunters who were reluctant to allow such an incision in the pectoralis muscles of their birds allowed the removal of a wing from which samples were taken instead. All samples were fixed in 10% neutral buffered formalin, embedded in paraffin using standard histological

techniques, sectioned at 6-10 μm, and stained with Harris' hematoxylin and eosin for routine examination for microcysts of *Sarcocystis*.

A bird was recorded infected with sarcosporidia if macrocysts were visible in the flesh, or microcysts were detected during histological examination. One cross section of the tissue having an area between one and two square centimeters was scanned at 100X. All birds were considered to constitute part of a random sample with respect to the presence or absence of sarcosporidia except those macroscopically infected birds submitted by hunters. These latter birds were not included in the survey results, but were used for feeding experiments.

As a check on the reliability of the method of diagnosis described above, a second piece of tissue was removed from the samples for each of 125 birds and prepared for histological examination. This duplicate series, which was selected randomly and independently of previous diagnosis of *Sarcocystis*, included samples from 40 blue grouse, and 85 sampples from other birds of various species. In addition, samples from the muscles of the wings, pectoralis complex, back, and legs of the two infected blackbirds were sectioned to determine if there was any site preference by the parasite.

For careful study of the cyst wall, additional sections cut at 2-3 μm were made of tissues found infected by the above method. To demonstrate the wall structure, the thin sections were stained with either of Harris' hematoxylin and eosin, Heidenhain's iron hematoxylin, or Gomori's reticulin silver. Photographs were taken with a Zeiss photomicroscope using Kodak Panatomic-X film.

Statistical analysis of the prevalence data obtained was calculated from 2 x N contingency tables using the χ^2 test, or the G statistic

described in Sokal and Rohlf (1969). The values obtained with the G statistic closely approximate χ^2 values, and are easier to compute when the contingency tables have more than two rows and columns (Sokal and Rohlf, 1969). Levels of significance for the G value are read from χ^2 tables for the appropriate degree of freedom.

Attempts to elucidate the life cycle(s) of avian *Sarcocystis* were made by *ad libitum* feeding of sarcocyst-infected flesh, or by force feeding infected flesh to selected mammalian carnivores. These included dogs, cats, ferrets, mink, coyotes, and rats. The flesh was established as infected by observation of macrocysts, or by using the method described by Fayer and Kocan (1971) for the presence of microcysts. It consists of grinding some meat and then examining under the microscope a few drops of the tissue exudate. The presence of endozoites in the fluid is indicative of sarcocysts in the flesh.

The dogs were beagle pups reared coccidia-free within the University Bioscience Animal Services (BAS). The cats came from the Department of Psychology and the kittens were from litters reared coccidia-free for this study in BAS. Coyotes came to the University from the wild as pups, and were housed in BAS upon their arrival. The rats came from a colony maintained by the University. The mink, purchased from a commercial fur ranch near Edmonton, were probably not coccidia-free when received. Fecal examination at the time of purchase did not reveal coccidian oocysts, but one mink began passing oocysts of *Isospora* sp. and *Eimeria* sp. shortly after it was bought. The ferrets were purchased from a commercial supplier in New York who had been given instructions that the animals should be freshly weaned, and that they were never to have been fed flesh.

When received, all of the ferrets were infected with an unidentified *Isospora*. To prevent possible cross-infection with other animals they were housed in a room by themselves. They were treated unsuccessfully with Nitrofurazone for one week, and then successfully with sodium sulfamethazine. Ninety-six hours were allowed to elapse after drug removal before the first feeding experiment began.

The animals and birds were housed in several rooms in the BAS Center. Each room was cleaned daily by an animal technician who had been given special instructions to minimize transmitting unwanted coccidians from room to room. The carnivores never occupied the same room as the experimental intermediate hosts. The ducks used in experiments 3 and 5 were obtained from a lot hatched and reared at the University which previous to this work had been held in a barn at the University farm. Those used in Experiment 13 were obtained as eggs from nests in the wild, brought into BAS where they were incubated, hatched, and reared. Chickens and Japanese quail were similarly reared from eggs in the Center. All experiments were conducted in the BAS Center. None of the experimental animals or birds were on medicated food at the time of the experimental feedings.

Fifty-seven black-billed magpie eggs were removed from nests and brought into the laboratory for hatching and rearing of *Sarcocystis*-free birds. Of the half that hatched, none were reared successfully. Similarly, nestlings brought into the laboratory were not reared easily. All were infected with an unidentified *Isospora* which seemed to be quite pathogenic to the birds. The eggs and nestlings were kept in a different room from the ducks and Japanese quail as a precaution against accidental infection spreading to other test birds.

Fecal samples were collected from all experimental animals, irregularly before the feedings of sarcocysts, and daily or semi-daily thereafter. Microscopic examination for the presence of oocysts or sporocysts was done at 200X and at 400X under phase contrast after concentration in sugar. Isosporan oocysts from kittens fed black-billed magpie flesh infected with *Sarcocystis* microcysts were cleared of fecal debris by screening through a 100-mesh sieve, concentrated by centrifugation in a zinc sulfate solution, and washed and stored in 2.5% potassium dichromate at 4° C. For the experimental infection of intermediate hosts, the dichromate was washed out, concentration of the resulting suspension determined with a haemocytometer, and known numbers of oocysts fed *per os** to laboratory hatched and reared pintail ducklings and Japanese quail. Details of the specific feeding experiments are given with the results for these experiments.

**Per os* refers to the technique of introducing substances directly into the stomach of an animal.

3. RESULTS

Prevalence

Nine hundred birds were examined macroscopically, and 885 of these were examined microscopically for the presence of sarcocysts. The results of this survey are given in Tables 1 to 6. Birds, grouped in orders and families, are classed according to age. Any bird approaching one year of age or more is placed in the adult category, while birds less than eight months of age are included in the juvenile category. Samples from a number of birds of undetermined age were also obtained; they are included in the category "age unknown." Since sex data often were not available, results are not categorized according to sex.

Of the 125 duplicate samples prepared histologically for *Sarcocystis* examination, 121 gave the same result both times. Two samples diagnosed positive the first time were negative the second, and two that were negative the first time were positive the second.

Muscles from the breast, back, both wings, and both legs from two infected blackbirds (one red-winged, and one yellow-headed) were examined for the presence of sarcocysts. In all, 71 separate samples were taken and examined. Nine of 10 from the pectoralis muscles, 31 of 31 from the legs, 14 of 14 from the wings, and 14 of 16 from the back contained cysts. The back samples, however, were very small pieces of tissue compared to those of other muscle groups. As a result of the apparent distribution of the cysts throughout the striated musculature, and the very similar results in the duplicate series, diagnosis of infection with *Sarcocystis* by the histological technique is considered reliable.

Examination of Tables 1 to 6 indicates that where ages are known, only adult birds were infected. Some birds of unknown age were also infected, but this latter category probably includes adults as well as juveniles. Samples for individual species frequently were not large, but it is apparent that adults of some species were more frequently infected than those of other species. Nearly half the adult shovelers and a third of the adult white-winged scoters were parasitized (Table 1) while members of the genus *Anas* (excluding shovelers) were less frequently infected.

The results of the statistical test (G statistic) for the prevalence data are given in Table 7. Juveniles were not included in the calculations because they are very rarely infected (Chabreck, 1965; Hoppe, 1976), nor were those species with very small sample sizes (less than five) included. The tests were performed on related groups, e.g., within the anatids, and within the corvids. These results show that differences in prevalence between the species are statistically significant. A comparison of the survey results of Chabreck (1965) and Hoppe (1976) with those found in this study is given in Table 8.

Sample sizes for species in Tables 2, 3, and 5 were very small, thus statistical comparison of the prevalence data for them would not be valid. Nonetheless, the finding of 2 of 6 California gulls infected might indicate a fairly high prevalence in this species. Similarly, the strigids appear to have a relatively high prevalence of sarcosporidia since nearly half the snowy owls and great horned owls were infected. Neither of the two hawks examined were infected, but 2 of 2 goshawks collected in southern British Columbia were infected (W. R. Salt, pers. comm.). In addition, while it gives little indication of prevalence, the

Table 1. Prevalence of infection with Sarcosporidia in some members of the Order Anseriformes. *

	Adult	Juvenile	Age unknown	Total
Family Anatidae				
American widgeon	0/2 **	0/10	0/4	0/16
Blue-winged teal	0/2	0/5	0/2	0/9
Gadwall	1/8(12)	0/3	-	1/11(9)
Green-winged teal	0/3	0/11	-	0/14
Mallard	3/99(3)	0/36	3/129(2)	6/264(2)
Pintail	5/52(10)	0/16	1/15(7)	6/83(7)
Shoveler	8/17(47)	0/14	0/7	8/38(21)
Mallard x pintail hybrid	-	0/1	-	0/1
Canvasback	-	0/3	0/1	0/4
Lesser scaup	3/36(8)	0/19	-	3/55(5)
Redhead	-	0/7	0/2	0/9
Bufflehead	-	-	0/2	0/2
Common golden eye	0/1	0/1	-	0/2
Ruddy duck	0/1	-	-	0/1
White-winged scoter ***	5/15(33)	-	1/1	6/16(38)
Whistling swan	-	-	0/2	0/2

* In some species, both macrocysts and microcysts were found. The gadwall was infected with macrocysts, while both macrocysts and microcysts were found in mallards, pintails, and shovelers (though not in the same individuals).

** Number of birds infected/number of birds examined (per cent infected)

*** *Sarcocystis* previously unrecorded in this host.

Table 2. Prevalence of infection with Sarcosporidia in some members of the Orders Gaviiformes, Podicipediformes, Ciconiiformes, and Gruiformes.

	Adult	Juvenile	Age unknown	Total
Family Gaviidae				
Common loon	-	0/2	-	0/2
Family Podicipedidae				
Red-necked grebe	-	0/1	0/6	0/7
Western grebe *	-	-	1/4	1/4
Family Ardeidae				
American bittern	-	-	0/1	0/1
Great blue heron	-	-	0/1	0/1
Family Rallidae				
American coot	-	-	0/18	0/18

* *Sarcocystis* previously unrecorded in this host.

Table 3. Prevalence of infection with Sarcosporidia in some members of the Order Charadriiformes.

	Adult	Juvenile	Age unknown	Total
Family Scolopacidae				
Common snipe	-	-	1/11(9)	1/11(9)
Greater yellowlegs	-	-	0/1	0/1
Lesser yellowlegs	-	-	0/1	0/1
Pectoral sandpipper	-	-	0/1	0/1
Short-billed dowitcher	-	-	0/9	0/9
Willet	-	-	0/1	0/1
Family Laridae				
Black tern	0/4	-	-	0/4
Bonaparte's gull	-	-	0/1	0/1
California gull *	-	-	2/6(33)	2/6(33)
Franklin's gull	0/4	-	0/6	0/10
Ring-billed gull	0/1	0/1	0/1	0/3

* *Sarcocystis* previously unrecorded in this host.

Table 4. Prevalence of infection with Sarcosporidia in some members of the Order Galliformes.

	Adult	Juvenile	Age unknown	Total
Family Phasianidae				
Ring-necked pheasant	-	-	0/4	0/4
Family Tetraonidae				
Blue grouse	3/52(6)	-	-	3/52(6)
Ruffed grouse	-	-	0/1	0/1
Sage grouse	-	-	1/1	1/1
Sharp-tailed grouse	1/19(5)	0/57(0)	-	1/76(1)
Willow ptarmigan * #	-	-	1/16(6)	1/16(6)

* *Sarcocystis* previously unrecorded in this host.

The ptarmigan were collected from the Northwest Territories by a student (Mr. R. Decker) who examined the birds macroscopically for the presence of *Sarcocystis*. A sample of the infected bird was brought to me for identification. Samples from birds not macroscopically infected were not kept.

Table 5. Prevalence of infection with Sarcosporidia in some members of the Orders Falconiformes and Strigiformes.

	Adult	Juvenile	Age unknown	Total
Family Falconidae				
Copper's hawk	-	-	0/1	0/1
Swainson's hawk	-	-	0/1	0/1
Family Strigidae				
Boreal owl	-	-	0/1	0/1
Great grey owl	-	-	0/3	0/3
Great horned owl	-	-	5/12(42)	5/12(42)
Snowy owl *	-	-	4/10(40)	4/10(40)

* *Sarcocystis* previously unrecorded in this host.

Table 6. Prevalence of infection with Sarcosporidia in some members of the Order Passeriformes.

	Adult	Juvenile	Age unknown	Total
Family Corvidae				
Black-billed magpie *	23/36(64)	0/2	-	23/38(61)
Common crow *	5/31(16)	0/1	-	5/32(16)
Grey jay *	4/10(40)	-	-	4/10(40)
Family Turdidae				
American robin	-	-	0/2	0/2
Family Bombycillidae				
Bohemian waxwing	-	-	0/6	0/6
Family Sturnidae				
European starling	0/1	-	-	0/1
Family Icteridae				
Brewer's blackbird	0/1	-	-	0/1
Brown-headed blackbird	0/1	-	-	0/1
Red-winged blackbird	2/12(17)	-	-	2/12(17)
Yellow-headed blackbird	1/1	0/1	-	1/2
Family Fringillidae				
Evening grosbeak	-	-	0/1	0/1
Hoary redpoll	-	-	0/1	0/1
Snow bunting *	-	-	1/1	1/1

* *Sarcocystis* previously unrecorded in these hosts.

Table 7. Calculated G test values from 2 x N contingency tables for selected groups of birds.

Species compared	G test Values	D. F.*	Significance level
Gadwall Mallard Pintail Shoveler	18.047	3	P < .005
Gadwall Mallard Pintail Lesser Scaup Shoveler White-winged Scoter	28.348	5	P < .005
Lesser Scaup White-winged Scoter	4.562	1	P < .05
Blue Grouse Sharp-tailed Grouse	.008	1	N. S. **
Black-billed Magpie Common Crow Grey Jay	16.592	2	P < .005

* D. F. = Degree of Freedom

** N. S. = Not Significant

Table 8. Prevalences of sarcosporidiosis in anatids from three surveys in North America.

Species	Chabreck (1965)	Hoppe (1976)	This study	G test Value	Level of Significance
Blue-winged Teal	38/79(48)*	7/85(8)	0/2	-	-
Gadwall	5/55(9)	0/45(0)	1/8(12)	3.43	N. S.**
Green-winged Teal	9/19(48)	2/42(5)	0/3(0)	-	-
Mallard	68/250(27)	24/307(8)	3/99(3)	27.369	P < .005
Pintail	162/365(44)	6/54(11)	5/52(10)	23.697	P < .005
Shoveler	36/46(78)	15/45(31)	8/17(47)	9.914	P < .01
American Widgeon	14/61(23)	3/49(6)	0/2(0)	-	-
Lesser Scaup	4/23(17)	0/46(0)	3/36(8)	4.765	P < .05
Redhead	-	0/37	-	-	-
Golden Eye	-	0/19	0/1	-	-
Bufflehead	-	0/12	-	-	-
Ruddy Duck	-	0/13	0/1	-	-
White-winged Scoter	-	-	5/15(33)	-	-

* Number infected/ number examined (percent infected)
** N. S. = Not Significant
Values for adult birds only included in the calculations.

infected western grebe was the most intensely parasitized bird found in the entire study. Almost every microscope field at 100X contained a microcyst compared with only one to a few microcysts per tissue section for most other infected birds.

In mammals, sarcocysts are frequently found in cardiac tissue, but hearts from a shoveler, 27 mallards, 27 pintails, 53 blue grouse, and 4 red-winged blackbirds did not contain sarcocysts. Examination of skeletal muscles of these same birds revealed that the shoveler had macrocysts, while one mallard, one pintail, three blue grouse and one red-winged blackbird each had microcysts.

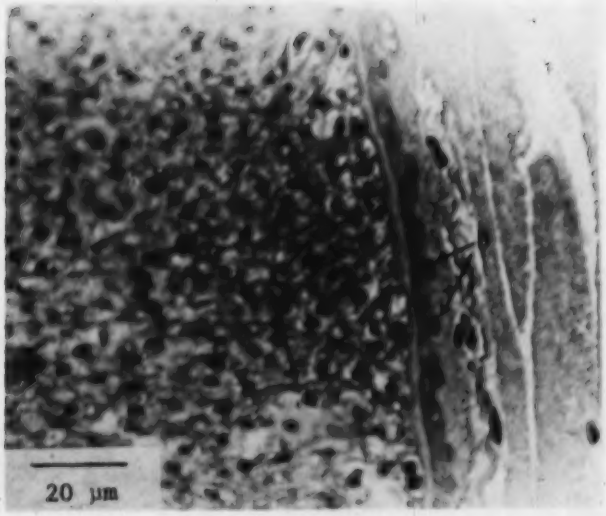
Structure of the Sarcocyst

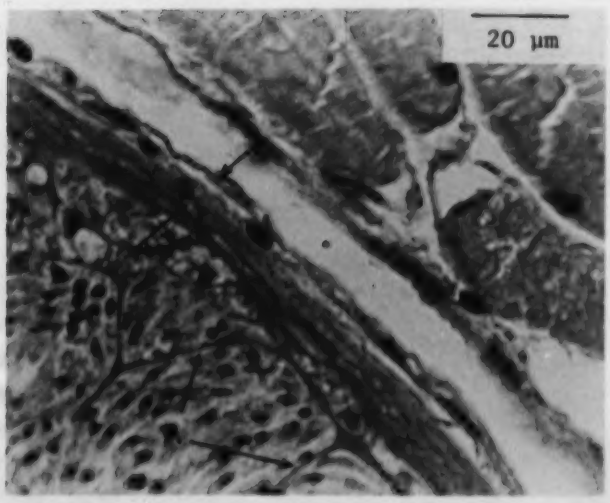
The walls of macrocysts from ducks were structurally similar to those of macrocysts of *Sarcocystis "tenella"* from sheep described by Senaud (1967). The outer surface of the primary wall of some macrocysts was elaborated into short club-like extensions (Plate 1) projecting outwards. These club-like processes are very similar to Senaud's "cauliflower" structures. Other macrocysts lacked the protrusions on the outer surface of the primary wall; they were surrounded by a layer of argyrophylic connective tissue between them and the surrounding muscle cells (Plate 2). The primary wall in macrocysts did not take up the silver stain. The interior of all macrocysts was divided into chambers by septa which arose from the inside of the primary cyst wall (Plates 1 and 2).

Characteristic and easily recognizable differences in the structure of the cysts, especially among the microcysts, were found. These differences apparently did not depend upon the host species infected, since

Plate 1. Thin (2 μm) section through a macrocyst from a gadwall. Septa (A) divide the cyst into chambers, and "club-shaped" protrusions can be seen (B) on the outer surface of the cyst wall. Harris' hematoxylin and eosin.

Plate 2. Thin (2 μm) section through a macrocyst from a pintail. Septa (A) divide the cyst into chambers, "club-shaped" structures are absent from the cyst wall, and the secondary wall (B) can be seen very closely applied to the primary cyst wall. Harris' hematoxylin and eosin.


20 µm


20 µm

similar cyst types were found in several unrelated host species, and often the same host individual harbored more than one cyst type.

Two basic types of cyst wall were found for microcysts. One type (hereafter referred to as Type 1) is characterized by a smooth surface on the primary wall (Plates 3 to 6), while the second type can be recognized by the presence of digitiform projections on the outer surface of the primary wall (Plates 7 to 9) (hereafter referred to as Type 2). Based on the thickness of the cyst wall and on the arrangement and degree of compaction of the endozoites in the cyst's interior, Type 1 cysts are divided into four subtypes (Plates 3 to 6). On the basis of the length and proximity of the projections, and the internal arrangement of endozoites, Type 2 cysts are subdivided into three subtypes (Plates 7 to 9). The following is a summary of the characteristics of each of the microcyst types and subtypes:

Type 1 Microcysts

1a - cyst wall very thin (less than 0.3 µm)
- outside contour of the cyst wall smooth
- endozoites moderately packed
- internal septa not easily distinguishable, or absent

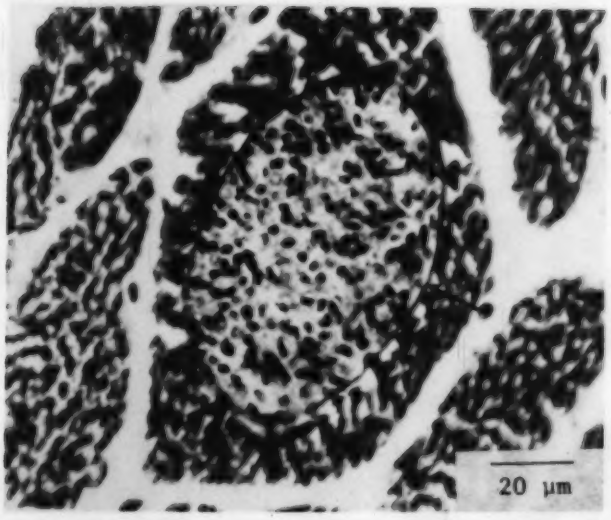
1b - similar to 1a, but endozoites very densely packed

1c - cyst wall recognizably thicker (about 0.6 µm)
- septa usually readily visible
- endozoites more loosely packed than in 1a

1d - as in 1b but outer contour of cyst wall presenting an irregular or "wrinkled" appearance
- septa readily visible

Plate 3. Microcyst of Type 1a found in the flesh of a black-billed magpie. The cyst wall (arrow) is very thin, and the endozoites are moderately packed. Heidenhain's iron hematoxylin.

Plate 4. Microcyst of Type 1b found in the flesh of a pintail. The cyst wall (arrows) is very thin, but in this cyst type the endozoites are densely packed. Harris' hematoxylin and eosin.


20 µm

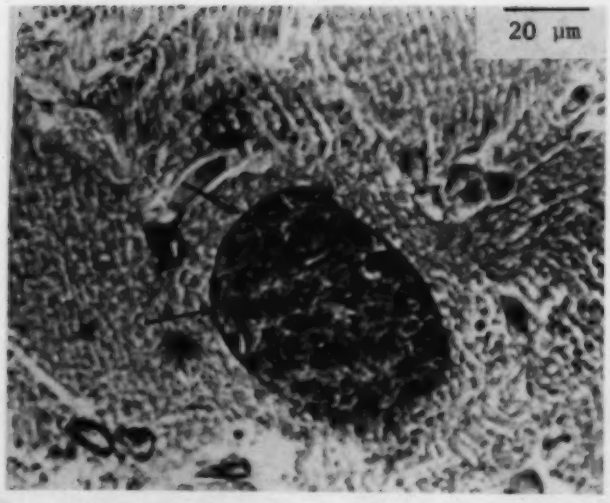
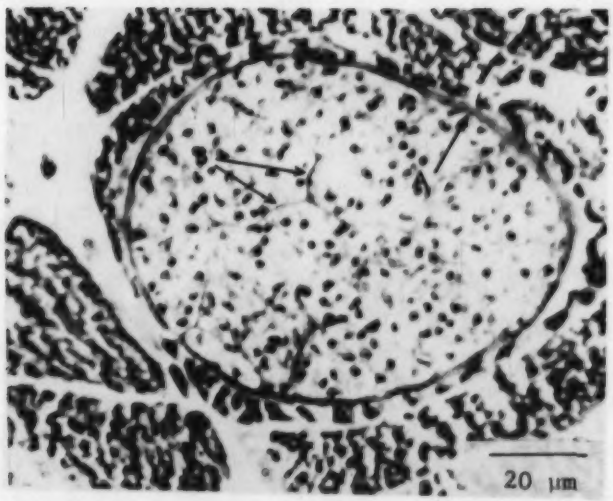

20 µm

Plate 5. Microcyst of Type 1c found in the muscles of a black-billed magpie. Note thicker cyst wall (A), internal septa (B) dividing the cyst into compartments, and loosely packed endozoites. Heidenhain's iron hematoxylin.

Plate 6. Microcyst of Type 1d found in the muscles of a great horned owl. The cyst wall (A) is very thin and wrinkled, internal septa (B) are present and readily distinguishable, and the endozoites are densely packed. Harris' hematoxylin and eosin.


20 µm

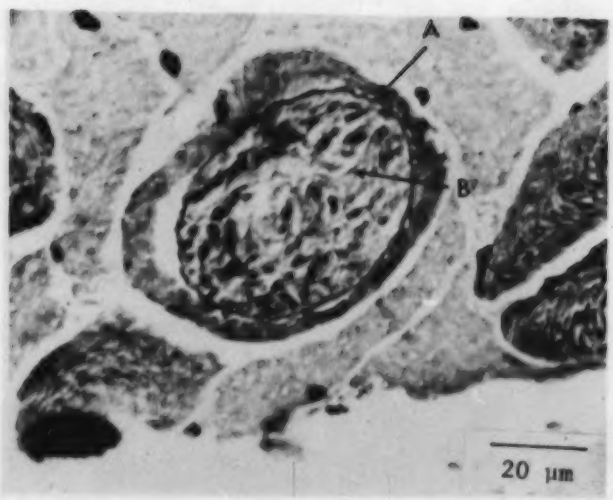
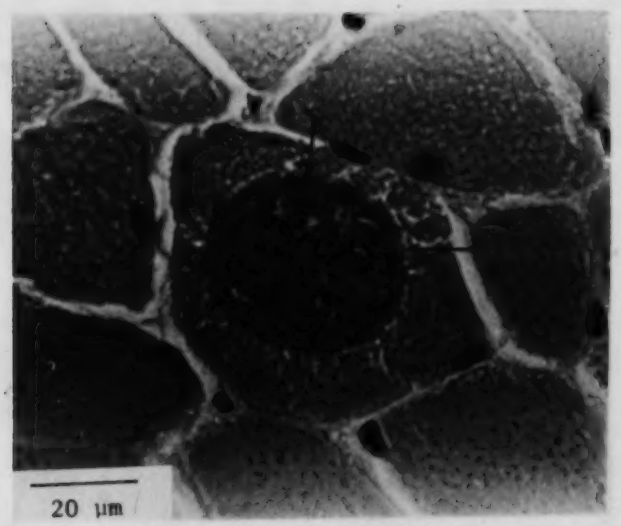

A
B
20 µm

Plate 7. Microcyst of Type 2a found in the muscles of a black-billed magpie. The fringe of numerous villi surrounding a thin cyst wall (arrows), and the dense arrangement of endozoites are characteristic. Harris' hematoxylin and eosin.

Plate 8. Microcyst of Type 2b found in the muscles of a pintail. The villi (arrows) are much less numerous and more distantly spaced; and the endozoites are loosely packed. Heidenhain's iron hematoxylin.


20 µm

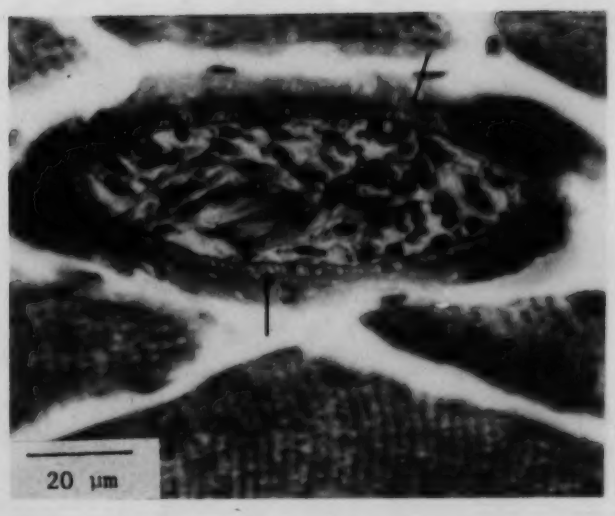
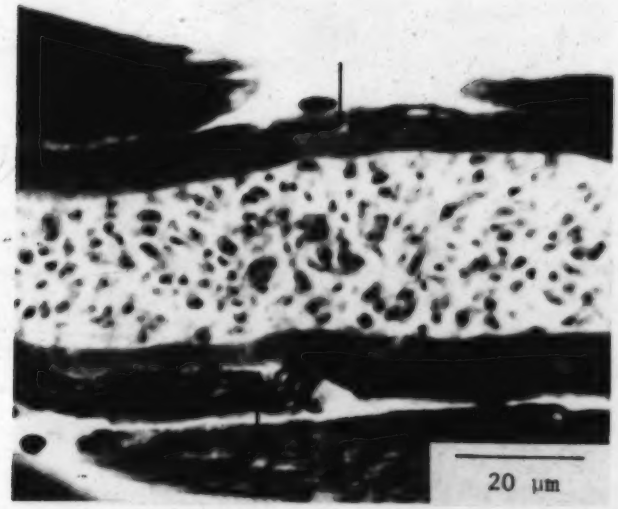
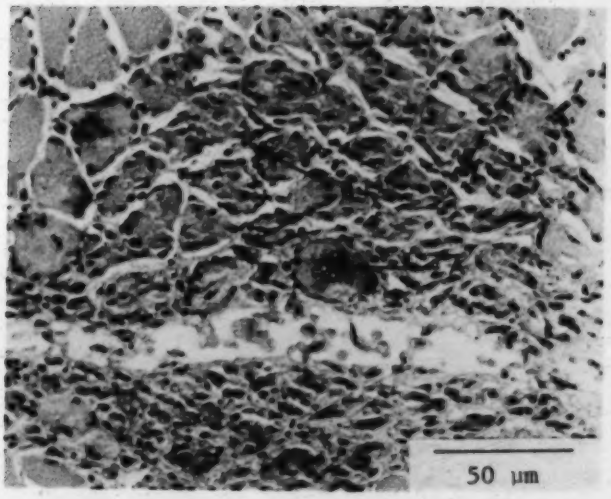

20 µm

Plate 9. Microcyst of Type 2c in longitudinal section from the muscles of a mallard. The length of the villi (arrows) and loosely packed endozoites are readily visible. Heidenhain's iron hematoxylin.

Plate 10. Pathological host response in the vicinity of a microcyst (A). Note the leucocytic infiltration (B). Harris' hematoxylin and eosin.


20 µm


50 µm

Type 2 Microcysts

2a - as in 1a, but radial spines present and quite close together, numerous (about 2 µm long)

2b - as in 2a, but radial spines noticeably fewer and more distantly spaced

- endozoites more loosely packed than in 2a

2c - cyst wall thin (less than 0.3 µm)

- radial spines numerous, long (4-5 µm), "shaggy" in appearance

- endozoites loosely spaced

Table 9 lists the birds infected with microcysts and shows the numbers of individuals having each kind of microcyst. The number of birds shown infected differs from the totals of infected birds presented in Tables 1 to 6 for three reasons. Firstly, the normal lower working range for paraffin sections is usually considered to be 4 to 6 µm; to cut at two is to push the method to its technical limit. Not all tissues were fixed in near perfect condition; therefore it was impossible to resection all infected tissues at 2 µm. The second reason was that thin sections of tissues earlier shown to be infected did not always contain sarcocysts. Some tissues cut at 2 µm cut very poorly and thus only fragments of the sections could be mounted on slides. Finally, tissues from some individual birds occasionally contained more than one type of cyst. Those birds having more than one cyst type are listed in Table 10.

Examples of cyst-associated pathology were few; nonetheless, two examples were found. In Plate 10 a microcyst is shown surrounded by an intense host inflammatory response and necrosis of nearby muscle fibres.

Table 9. The types of microcysts found in each bird species.

Species	Cyst Type 1				Cyst Type 2			Total * Cyst Types
	1a	1b	1c	1d	2a	2b	2c	
Mallard	-	-	-	-	-	1**	1	2
Pintail	3	2	1	-	-	-	-	3
Shoveler	-	1	-	-	-	-	-	1
Lesser Scaup	-	-	1	-	-	2	-	2
White-winged scoter	-	1	-	-	1	3	1	4
Western grebe	1	-	-	-	-	-	-	1
Blue grouse	2	-	-	-	-	-	-	1
Willow ptarmigan ***	-	1	-	-	-	-	-	1
Wilson's snipe	-	-	-	-	1	-	-	1
California gull	-	-	1	1	-	-	-	2
Great horned owl	2	2	2	-	-	-	-	3
Snowy owl	1	1	-	1	-	-	-	3
Common crow	1	1	-	1	1	1	-	5
Black-billed magpie	3	8	8	1	5	2	-	6
Grey jay	3	-	-	-	1	-	-	2
Red-winged blackbird	-	2	-	-	-	-	-	1
Yellow-headed blackbird	-	1	-	-	-	-	-	1
Snow bunting	-	1	-	-	-	-	-	1

* Total types of cysts found in each species.

** Number of individual birds infected with a particular cyst type.

*** Note: although the willow ptarmigan's cysts were visible macroscopically, the cyst wall was quite thin, and displayed typical microcyst wall structure.

Table 10. The types of cysts found within individual birds with multiple infections.

Species	Type of Cyst						
	1a	1b	1c	1d	2a	2b	2c
Pintail	+	+	-	-	-	-	-
Pintail	+	-	+	-	-	-	-
Pintail	+	+	-	-	-	-	-
Lesser Scaup	-	-	+	-	-	+	-
White-winged Scoter	-	-	-	-	+	+	-
California gull	-	+	+	-	-	-	-
Great horned owl	-	+	+	-	-	-	-
Black-billed magpie	-	-	+	-	+	-	-
Black-billed magpie	-	+	+	-	-	-	-
Black-billed magpie	+	+	-	-	-	-	-
Black-billed magpie	-	+	-	-	-	+	-
Black-billed magpie	-	+	+	-	-	-	-
Black-billed magpie	-	-	+	-	-	+	-
Black-billed magpie	-	+	-	-	+	-	-
Black-billed magpie	+	+	+	-	-	-	-
Common crow	+	+	-	+	-	-	-

It is not known whether the parasite provoked the host reaction (e.g., by leakage of cyst contents) or whether it was accidentally involved in a reaction to some other agent. Other cysts in this same bird had no pathological responses around them.

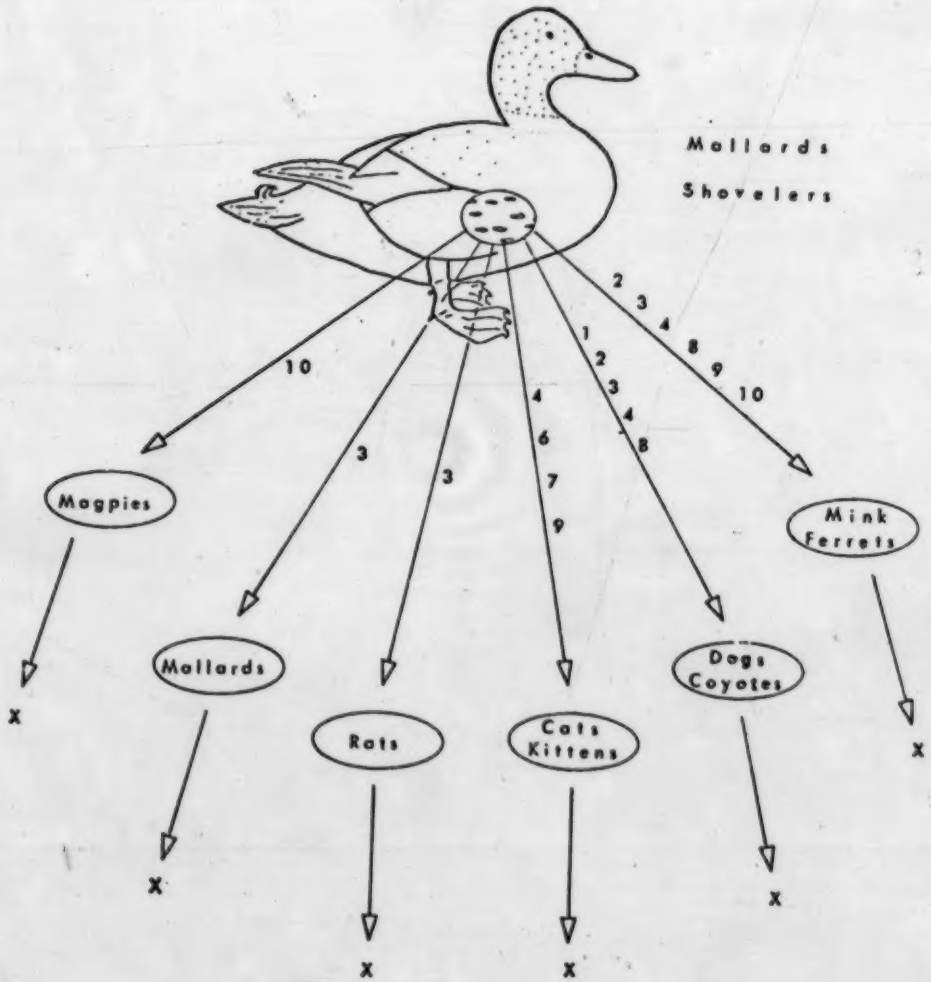
Many muscle samples had foci of inflammation characterized by necrosis of muscle fibres, infiltration of leucocytes, and somewhat less often, fibrous tissue formation. With the exception of the example discussed above, and one other example from a section of blue grouse muscle which had a similar, though less intense reaction around the cyst, the pathology found could not be associated with the presence of sarcocysts.

Life Cycle Experiments

During this study many attempts to elucidate the life cycle of avian *Sarcocystis* were made. While the major emphasis was to determine the cycle of the macrocyst-producing parasite (*S. "rileyi"*) from ducks by feeding cyst-infected meat to particular carnivore species, other indirect approaches to the problem also were made. Thus endozoites were injected subcutaneously in some ducks, and fed to other ducks. In addition, microcyst-infected flesh from a white-tailed deer was fed to a dog. Figure 1 summarizes the life cycle experiments attempted.

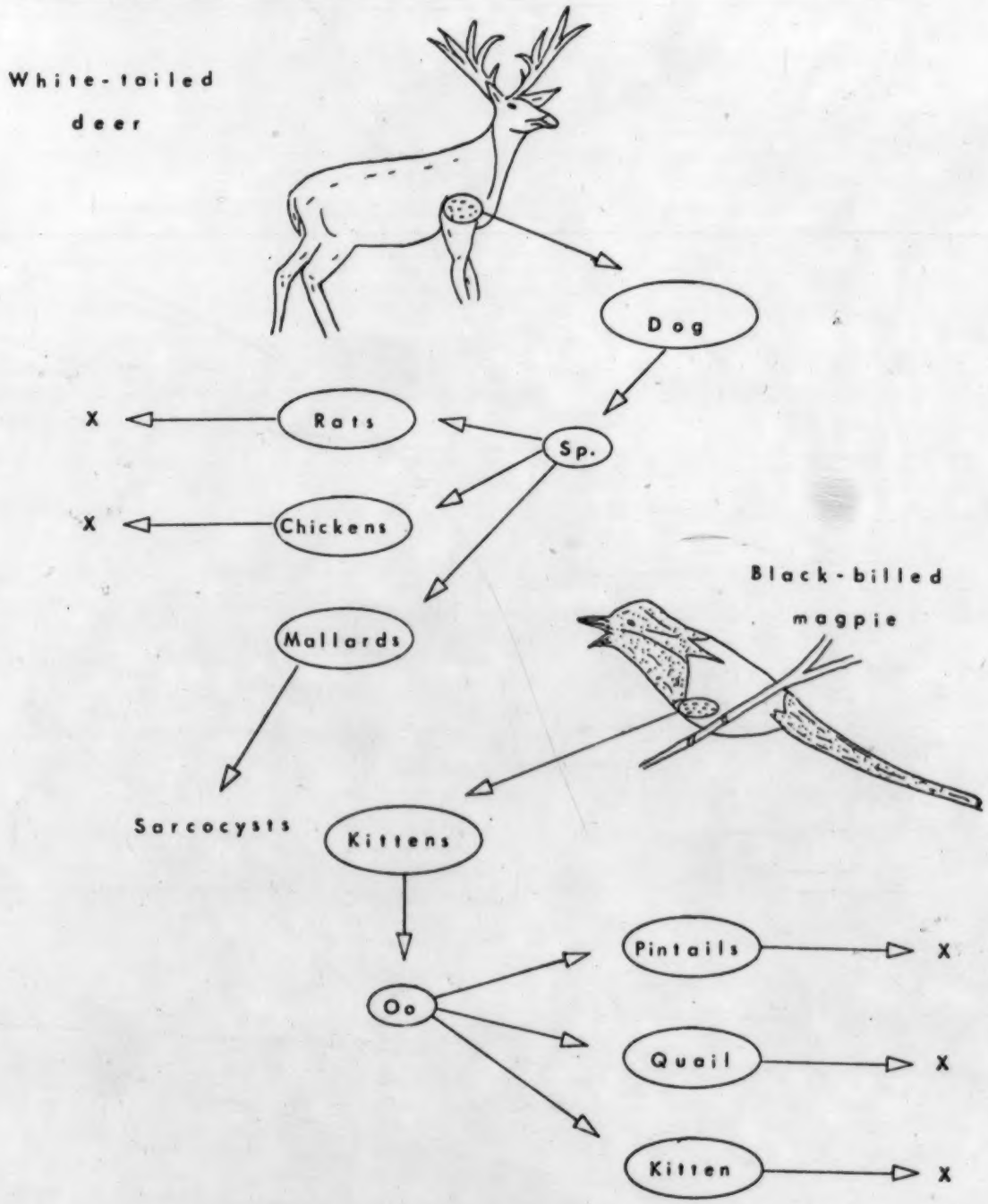
In a series of experiments during the autumn of 1974, four macroscopically infected mallards were submitted by hunters. The first of these (Experiment No. 1) was fed 48 hours port mortem to a six-month old beagle puppy. Feces examined every second day for 24 days did not contain oocysts or sporocysts. In Experiment No. 2, flesh from a freshly killed infected mallard was divided into three portions. A second beagle puppy, and an eight-month old coyote each received one of the three portions.

FIGURE 1. Summary of life cycle experiments with *Sarcocystis*.



Sketched animal represents source of sarcocysts
Numbers indicate number of individual experiment
X - indicates an unsuccessful experiment

FIGURE 1. Continued:



Sp. represents presence of sporocysts in the feces

Oo. represents presence of oocysts in the feces

Feces examined from each animal every second day for 24 days were negative.

The third portion was fed to an adult mink. Fecal samples were collected every second day. On day 6 the mink feces contained a few large, unsporulated oocysts (28.5 x 33.4 µm; N = 10) which upon sporulation were identified as *Isospora* sp. In addition, some very small (10-12 µm) spherical oocysts of *Eimeria* sp. were also passed coincidentally. Oocysts of both types were passed for another six days, i.e., up to day 12. After that time, no oocysts were passed. As this animal was not passing oocysts when purchased but began passing them very soon thereafter, it is believed it became infected about the time it was bought.

A very heavily infected mallard (turned in six days post mortem) was used in Experiment No. 3. The flesh was divided into five portions. Four portions were fed to experimental animals, i.e., two mink, a beagle puppy (the same one as was used in Experiment No. 1), and one portion to three white laboratory rats. Feces collected daily for 29 days from each of these animals did not contain coccidian oocysts or sporocysts.

From the fifth portion of infected flesh, about 100 macrocysts were removed and placed in Earle's saline solution. One-third of these cysts were disrupted by hand in a ground glass tissue homogenizer in sterile saline, and the resulting suspension injected subcutaneously into each of six mallards ($1\frac{1}{2}$ years old). Feces were collected for 29 days, but did not contain either coccidian oocysts or sporocysts.

The last portion of isolated macrocysts was disrupted as above in a solution of Earle's saline containing 10% dimethyl sulfoxide (DMSO). Following disruption, the resulting suspension was divided into aliquots in 2 ml vials and deep-frozen using the technique described by Dumas (1974) for *Toxoplasma gondii*.

The endozoites deep-frozen in Experiment No. 3 were used 90 days later in Experiment No. 6. Two vials were removed from the freezer and their contents rapidly thawed by placing the vials in luke-warm water. Microscopic examination of a drop of the fluid showed endozoites that had the appearance of endozoites from freshly killed ducks. The contents of these two vials were then fed to an adult cat by pouring them over a bowl of canned cat food which the cat readily ate. Feces collected daily for 21 days did not contain either coccidian oocysts or sporocysts.

Flesh from a lightly infected mallard (the last one received in 1974) was fed to a cat, a mink, and to a beagle puppy (the one that was used previously in Experiments Nos. 1 and 3). Feces were collected daily for 14 days from the dog, and for 21 days from the cat and the mink. Examination of the feces revealed neither oocysts nor sporocysts.

In Experiment No. 5, thigh flesh containing microcysts from a white-tailed deer was fed *ad lib* to beagle puppy No. 1 (used in previous experiments) after passing the meat through a meat grinder. The deer was established as infected by examination of fresh tissue exudate using the method described by Fayer and Kocan (1971). Ten days later a small number of sporulated sporocysts (15.8 x 10.3 µm, N = 10) appeared in the feces. Although slightly larger, they were morphologically similar to those of *S. "fusiformis"* described by Heydorn and Rommel (1972) and by Mahrt (1973). Though few in number, they were concentrated by flotation in sugar solution, washed free of the sugar by several washings in tap water, and stored in 2.5% potassium dichromate at 4° C. Because few sporocysts were recovered, small quantities were given *per os* to four mallards, and to five laboratory-hatched chicks. One inoculated mallard

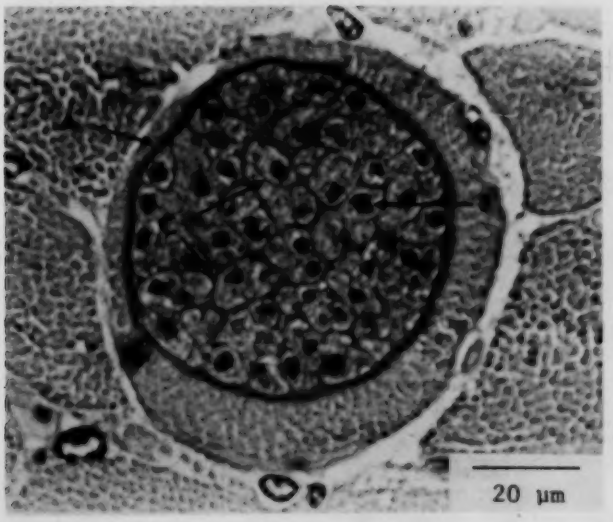
died within 24 hours, evidently as a result of having been pecked by its cagemates. Microscopic examination of a sample of its flesh did not reveal the presence of sarcocysts. Five weeks after infection one control duck died of unknown causes. Sarcocysts were not found in its flesh. Another week later a second inoculated duck died. Bruises on the side of its head and neck indicated it also was probably pecked to death. Flesh from this duck was fed to cat No. 1 and mink No. 4. Feces collected for 18 days did not contain oocysts or sporocysts. Histological examination of tissue from the pectoralis muscles did not reveal microcysts.

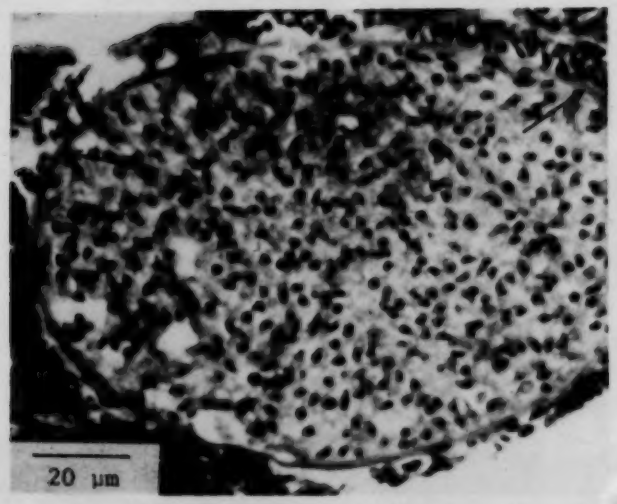
Ten weeks after infection the three remaining ducks (two inoculated and one control) were sacrificed. Cysts were not found in the control duck. Examination of the two inoculated ducks revealed the presence of a few small (40-60 μm) cysts containing zoites which, in one bird, appeared to be metrocytes (see Plate 11), and in the second test mallard, endozoites (see Plate 12). In both cases, the cysts were of Type 1c described in the preceding section. It is not known how these cysts compared to those present in the deer from which the flesh was taken since a tissue sample was inadvertently not kept. A thin section made later from a muscle sample of a mule deer revealed cysts similar to Types 1c, 2b, and 2c. This indicates the possibility that the cyst found in the mallards could have originated from the deer. None of the chicks had sarcocysts when examined 10 weeks later.

In the spring and early summer of 1975, another series of life cycle experiments was conducted under rigidly controlled conditions (except for the feeding experiment with black-billed magpie nestlings). The kittens were carefully reared to reduce the possibility of their becoming infected with any unwanted coccidian. The ferrets were equally

Plate 11. Microcysts in the muscles of a mallard infected with sporocysts from a dog fed infected deer flesh. The cyst wall (A) is of Type 1c and metrocytes (B) are present inside well-defined compartments (C). Harris' hematoxylin and eosin.

Plate 12. Microcysts in the muscles of a mallard fed sporocysts from a dog fed infected deer flesh. The well-defined cyst wall is of Type 1c and the endozoites are moderately packed. Septa are present, though not easily visible. Heidenhain's iron hematoxylin.


20 µm


20 µm

carefully housed after their arrival and treatment for the unwanted *Isospora* infection they were all infected with upon their receipt.

Five freshly killed shovelers infected with macrocysts were collected and fed to animals in Experiments 7 to 10. Each of three kittens was fed as much as it would eat (each had been starved for 24 hours). Feces were collected daily for 25 days from two kittens and 31 days from the third. Neither oocysts nor sporocysts were found.

Infected flesh was fed to each of four ferret kits, and to two adult mink. Feces collected and examined for 20 days from the ferrets and for 24 days from the mink were negative. Similarly, a coyote pup fed a portion of the cyst-infected flesh was not found to have either oocysts or sporocysts within 20 days.

Cyst-infected flesh from several of the shovelers was fed to six black-billed magpie nestlings (all infected from the wild with an unknown *Isospora* sp.) housed in a common cage. Six other magpie nestlings housed in a different cage (also infected from the wild with the same *Isospora* sp.) were not fed infected meat, but were used as controls. None of the nestlings was treated with a coccidiostat prior to the experimental feeding because the sarcocysts were available before treatment could be effected. Both groups continued to pass the *Isospora* sp. oocysts they had previously been infected with, but no oocysts or sporocysts different from those already present were passed. While oocyst output was not quantified, the *Isospora* sp. output of the test group of magpies appeared to increase while that of the control group remained at about the same level. Three test and one control birds died before the termination of the experiment; histological examination of tissues from these birds revealed massive intestinal hemorrhage and sloughing of intestinal

epithelium. The contaminating *Isospora* was believed responsible for the pathology in and subsequent deaths of these birds. Tissues of liver, spleen, brain, heart, and skeletal muscles appeared normal.

A final series of feeding experiments was conducted with microcysts originating in black-billed magpies. In Experiment No. 11, four freshly killed birds were brought into the laboratory; flesh from two was fed to one kitten while flesh from the other two was fed to a second kitten. Histological examination of the flesh of the birds revealed microcysts in both birds given to the first kitten, but microcysts were not seen in the flesh given to the second kitten. Beginning on day 7 and continuing until day 13, both kittens passed unsporulated oocysts which morphologically resembled those of *Isospora felis*. The kitten which received the known infected flesh passed many more oocysts than did the second kitten, although the actual quantities passed by each were quite low. Average size of the oocysts was 27.5 x 35.0 µm (N = 20), somewhat smaller than the average reported by Shah (1970) and Levine (1973), but well within the range. The oocysts sporulated in about 48 hours at room temperature.

In an attempt to increase the number of oocysts available for experimental infection of birds, 8,000 oocysts from the above experiment (No. 11) were administered *per os* to a kitten (previously fed shoveler sarcocysts, with no apparent infection). Feces were collected for 21 days, but no oocysts were passed after day 1 (unexcysted oocysts administered the previous day).

The final experiment in the series consisted of feeding the remaining oocysts (from Experiment No. 11) to each of two laboratory-

hatched and reared pintail ducklings, and four similarly reared Japanese quail chicks. Cagemates of the test birds served as controls. The pintails each received 6,000 sporulated oocysts while the quail each received 3,000. One control quail died the next day, apparently as a result of having been pecked by its cagemates. No sarcocysts were found in its tissues, either by histological examination, or by the method of Fayer and Kocan (1971). Two quail, one control and one test, were examined 55 days later for sarcocysts, but none were found. The remaining birds were sacrificed 98 days after infection. Examination of tissues by both methods did not reveal the presence of sarcocysts.

4. DISCUSSION

Prevalence

When this study was initiated, the newly reported life cycle of *Sarcocystis "tenella"* and *S. "fusiformis"* indicated that a carnivore-herbivore cycle was the usual method of transmission of the parasite. The simplicity of these two life cycles suggested that a life cycle for the macroscopic sarcocystid of ducks might be as easily elucidated. Thus the project began with the aim of quickly determining the life cycle of the duck parasite, and then continuing with laboratory investigations of some selected aspects of its biology. Failure to establish the life cycle resulted in greater emphasis being placed on other information, including the prevalence survey in avifauna, and the study of the cyst wall morphology with the light microscope.

The majority of the tissues examined were obtained from birds belonging either to other researchers, or from the Provincial Fish and Wildlife Division. All birds were dead when brought into the laboratory, and those from the Fish and Wildlife Division were frozen. As a result, the method of diagnosis of the birds for sarcocysts was necessarily limited to visual, i.e., gross and histological examination. Immuno-diagnosis may have been preferable, but the problems involved with the technique rendered it impracticable. These include having a source of antigen (from living endozoites), and having a usable amount of antibody.

Identification of sarcocysts in the birds did not present any problems. Once a person has seen macrocysts, recognition of them is a relatively simple matter. Tissues from birds with macrocysts were

prepared for histological examination, which confirmed the preliminary diagnosis. The presence of readily recognizable septa and endozoites inside the cysts is characteristic (as is the presence of cysts in striated muscle only). Microcysts were found after histological examination, and always exclusively in the interior of individual muscle fibres. Their intensely stained dark-blue appearance surrounded by the light orange-pink of eosin-stained unparasitized muscle fibres renders them very readily visible, even under low magnification (100X).

The problem was not one of identifying cysts, but one of finding the cysts in the first place. Since most birds having microcysts appeared to be lightly infected, what was the probability of a given tissue section not containing at least one sarcocyst? The duplicate set of prepared tissues did not reveal any significant difference between the two runs. The series of sections from different muscles from two infected blackbirds did not show any difference in probability of diagnosis as a result of using breast muscles for the routine examination, nor did it reveal that light infections were likely to be overlooked. Thus it seems that the technique is relatively reliable for disclosing the presence of microcysts (note the high number of black-billed magpies found infected in this way).

A characteristic location for *Sarcocystis* (at least some species) in mammals is cardiac muscle (Fayer and Johnson, 1973; Fayer, 1974). Erickson (1940) reported macrocysts in the myocardium of a pintail which had cysts in its skeletal muscles, but Fayer and Kocan (1971) did not demonstrate cysts in the hearts of 91 infected common grackles. All the hearts examined here were negative, even in birds with cysts in their skeletal muscles. Thus it seems that avian *Sarcocystis* does not normally develop in heart muscle, unlike some species of mammalian *Sarcocystis*.

This does not preclude the possibility that infection of the heart is so pathogenic that birds developing the parasite in the heart normally die and thus are not included in the sample. Only additional laboratory infection studies can clear up this question.

The highly significant differences in prevalence between the different anatids, and between the corvids, indicate that the parasite(s) is not distributed randomly among species. There is insufficient information available to fully explain the differences. Reasons for the differences might include different susceptibilities by different host species, and different probabilities of infection related to feeding ecology. Finding several cyst types (to be discussed more fully later) indicates that several parasite species may be present. Finding the same cyst types in unrelated hosts suggests a lack of host specificity. Since the route of infection presumably is oral, feeding ecology may be a major factor determining which species are most likely to be infected.

The available information indicates that both feeding ecology and host physiology are important in the acquisition of sarcosporidia by birds. There are no reports of macroscopically visible sarcosporidiosis in geese. Chabreck (1965) macroscopically examined "hundreds" but did not find it. Reports of sarcosporidia in American coots are rare, although they have occasionally been reported infected (Erickson, 1940). Since coots and geese are frequently present on the same waters as ducks, it is reasonable to suspect that they (the geese and coots) are also exposed to the macrocyst-producing *Sarcocystis* (assuming the infective oocysts/sporocysts are present in the water). Failure to find macrocysts in geese and coots suggests that they are more resistant to sarcosporidiosis.

The highly significant differences in the prevalence of infection between shovelers, mallards, pintails, and gadwalls, and between the corvids indicate that feeding ecology plays a major role in determining the frequency with which individuals of these species become infected. Physiological differences may also be involved in these hosts, but feeding ecology seems to be the more important here because of the relatively common occurrence of the parasite in these species. Only future experimental infections will demonstrate the role of host physiology, and any pathological response in determining the prevalence of infection in the different host species.

The prevalence of sarcosporidia in a given species may be depenent upon the geographical area where the survey was conducted. Comparison of Chabreck's (1965) and Hoppe's (1976) results with mine shows highly significant differences. These differences are all the more significant because microcysts were looked for only in this study. The three studies were conducted in widely separated areas: Louisiana (Chabreck, 1965), North Dakota (Hoppe, 1976), and Alberta (this study). Hoppe's results are essentially similar to mine, although my results include the presence of microcysts. If macrocysts only are counted, 2 of 52 pintails (4%) and 1 of 99 mallards (1%) were infected, results lower than Hoppe's. The significant differences calculated and presented in Table 8 would thus be even greater.

From Chabreck's (1975) report it is evident that the macrocyst-producing *Sarcocystis* of dabbling ducks is transmitted in Louisiana. Mottled ducks, which are resident all year in Louisiana, are infected with the parasite. Therefore, they must become infected there. Since mottled ducks acquire the infection in Louisiana, it is reasonable to suspect that

migratory species may also become infected there (a wintering ground for many migratory ducks).

Demonstrating acquisition of the parasite in one geographical area does not preclude its acquisition elsewhere as well. But this study indicates that the macrocyst-producing *S. "rileyi"* of ducks is not transmitted in Alberta. None of 14 juvenile shovelers collected in the fall were infected, but of 14 adult shovelers collected the following spring, 7 were infected, 6 with macrocysts. It is probable that many of these 14 adults were juveniles the preceding autumn. Hence it seems the period required for the appearance of macrocysts in shovelers is in the order of half a year, or less. Assuming that the juvenile sample of 14 from the fall (no adults were collected in the fall) can be compared with the 14 adults collected the following spring ($X^2_{[1]} = 9.333$, $P < .005$), it seems that this parasite is acquired on the host's wintering ground, not in Alberta. It also indicates that the growth period between time of infection and appearance of macrocysts is relatively short. This latter conclusion is supported by Chabreck's (1965) report of macrocysts in a juvenile shoveler.

If the above hypothesis (re: location of infection) is correct, then an explanation for the different results obtained in the three studies (Chabreck, 1965; Hoppe, 1976; this study) can be suggested: the birds examined in each study had different prevalences because they overwintered in different areas. Chabreck's birds were primarily Mississippi Flyway birds, and birds resident in Louisiana. The birds examined by Hoppe were primarily Central Flyway birds, and bulk of which overwinter along the Texas and the Mexican Gulf coasts (Linduska, 1964). The birds

I examined were predominantly Pacific Flyway birds which overwinter in the southwestern United States and along the Pacific Coast (Linduska, 1964; Poston, 1974). As a result of this, the specific ecological conditions in each overwintering area may affect the distribution of infectious stages (oocysts/sporocysts) in the environment. Thus the probability of ducks becoming infected in their respective wintering grounds will differ.

The above argument cannot be applied to non-migratory birds in which microcysts are found. Birds resident in Alberta must acquire *Sarcocystis* here if they are infected. Since it seems that *S. "rileyi"* is transmitted elsewhere, there must be a minimum of two species of *Sarcocystis* in the avian host.

The relatively high prevalences in each of the three corvid species examined (common crow, black-billed magpie, and grey jay) may be a reflection of their feeding ecologies. All are food generalists, feeding on whatever animal matter they come across, including the remains of predator kills. Ingestion of infectious oocysts or sporocysts would thus be a likely event. Their habits as scavengers may also make them potential definitive hosts for some species of *Sarcocystis*.

Pathology

Evidence concerning the possible pathogenicity of the parasite in birds is entirely lacking, but there is evidence that both pre-cystic and post-cystic (i.e., cyst degeneration) stages are pathogenic in mammals. The early reports reviewed by Scott (1943b) deal primarily with cyst degeneration. More recently, Spindler *et al.* (1946) reported illness in pigs during early stages of infection, and Fayer *et al.* (1973) showed

that calves fed sporocysts of *S. "fusiformis"* from dogs became ill, and often died. Clinical symptoms described by Fayer and Johnson (1973) include anorexia, cachexia, weight loss, anemia, and accelerated heart rates during the acute stages (pre-cystic). Post-mortem examination of these calves revealed hemorrhage of the serous membranes, pericardium, myocardium, dorsal surface of the cerebellum, and a generalized lymph-adenopathy (Fayer *et al.*, 1973). Investigation of possible pre-cystic pathology in the avian host was one of the objectives which depended upon successful determination of the life cycle, but had to be abandoned.

Recent papers by Mehlhorn *et al.* (1975a, b), Heydorn *et al.* (1975a, b) and Gestrich *et al.* (1975) suggest that cysts belonging to some species of mammalian *Sarcocystis* tend to be relatively short-lived. Generalizing these findings to the avian host leads to the expectation of finding evidence of degenerating cysts in some of the infected tissues examined. The fact that only two such examples were found may indicate either that the life span of cysts in birds is relatively long--i.e., approaching that of the bird--or that once cysts begin to break down the pathological response of the host to the liberated material is fatal to the bird. The fact that this has not been observed in birds may be because carcasses of birds that died from "natural" causes are seldom found (Lack, 1954). It may also indicate that any histopathological evidence was not recognized. Foci of inflammation and buildup of fibrotic tissue were seen frequently, but it was not possible to associate this with the presence of cysts.

Structure of the Sarcocyst

The limited available evidence (Kaliner, 1975; this study) shows

that there are a variety of cyst types which may be found in any given host species, and that the same cyst types can occur in different host species. Thus cyst structure may be relatively independent of host physiological differences and may be a relatively constant feature. Consequently, cyst morphology may be a useful taxonomic criterion.

A comparison of the cyst wall of duck macrocysts with the cyst wall of macrocysts of *S. "tenella"* described by Senaud (1967) does not indicate much difference between the two, other than size. Duck macrocysts are invariably smaller than the maximum reported for sheep sarcocysts, seldom exceeding 5 mm in length, and 2 mm in width. The cauliflower-like protrusions or regressions of the cyst villi reported by Senaud in young macrocysts were also visible in some duck macrocysts (Plate 1). In other ducks the cyst wall was a smooth complex of primary and secondary wall within which structures could not be observed (Plate 2). Senaud (1967) suggests that this is a function of age of the cyst. Without additional evidence from experimental infections, it is impossible to know if age alone is responsible for the "cauliflower" protrusions in duck macrocysts. It is not known if more than one parasite species is involved, or if development differs in different hosts.

In addition to the similarities described above, they are similar in another respect. The endozoites of *S. "tenella"* are 10 to 15 µm long and 3 to 4 µm wide (Scholtyseck *et al.*, 1973) and those of *S. "rileyi"* are 9.5 to 16 µm long and 2 to 3 µm wide (this study). In other words, on the basis of morphology at the light microscope level, cysts from the two groups cannot be distinguished. The only evidence that they represent distinct species lies in their life cycles. *S. "tenella"* develops in the

intestine of domestic cats (Rommel *et al.*, 1972) and of dogs (Munday *et al.*, 1975) but *S. "rileyi"* develops in neither (this study).

Recent work (Mehlhorn *et al.*, 1975a, b; Heydorn *et al.*, 1975a, b; Gestrich *et al.*, 1975) has shown that oocyst/sporocyst morphology are not taxonomically useful by themselves, but that when combined with definitive host information (i.e., definitive host species) their taxonomic usefulness is increased. More important, however, is their finding that the sarcocysts of the species of the *S. "fusiformis"* complex can be differentiated from each other at all stages of their development by morphological criteria. Some of these criteria are visible with the light microscope, and include length of radial villi, density or arrangement of both spines and endozoites, and apparent absence of villi in one species of the complex.

Scott (1943a) reported that as sheep age, the numbers of cysts associated with the first seasons of exposure declined while the numbers of cysts associated with recent infection remained high. He based this explanation on the sizes of various cysts, the older ones being larger. With each passing year the number of cyst size categories increased and the numbers of larger (hence older) cysts declined. An alternative explanation to Scott's hypothesis is that more than one species of *S. "tenella"* (as suggested by Munday *et al.*, 1975) is present in the sheep.

Evidence to assess the effect of cyst age on its structure was not obtained, but 16 birds were found infected with two or more types of cyst. If these cyst types belong to the same parasite species, then the only reasonable explanation must be one of different cyst ages; i.e., the host was infected more than once. Some mammalian species of *Sarcocystis* have been shown to grow to a maximum diameter and then stop

increasing their diameter (Mehlhorn *et al.*, 1975a). These same workers have also shown that morphological structures visible with the light microscope did not change appreciably during growth of the parasites. Since, in some birds with multiple infections, the different cyst types were the same size, the differences do not appear to be due to different ages of the cysts. This is supported by the fact that there was no apparent relationship between cyst type and cyst size among microcysts; i.e., that cysts of each type occupied a range of sizes. Thus the effect of different host species on the development of the cyst seems unimportant.

Cyst Type 1c was easily distinguishable from the other microcyst types on the basis of its thicker cyst wall which appeared to have an inner membrane as well as an outer one (Plate 5). Internal septa were usually visible, unlike in Types 1a and 1b. Mehlhorn *et al.* (1975b) have shown that in one kind of cyst in calves, originating from the large form of *Isospora bigemina* of dogs, the cyst walls appeared smooth when examined with the light microscope; but when examined with the electron microscope were found to have a very thin wall surmounted by regularly alternating long and short "clubs". The entire thickness of this complex was no more than 0.6 μm. As the cysts matured, the clubs became longer, but the cyst wall retained its thin appearance because these protrusions became folded over. Perhaps the "double-layered" appearance of the cyst in Type 1c has a structure similar to that described by Mehlhorn *et al.* (1975b). Demonstration of this will have to come from additional study of this avian cyst type with the electron microscope.

Cysts of Type 1d were not frequently found. The wrinkled appearance might be an artifact, but for the following reasons it is retained as a distinct type. The cyst wall is thin and therefore not of Type 1c.

In Type 1a septa are faintly, or not visible at all. In Type 1d septa are easily visible. In Type 1b, the endozoites are tightly packed, while in Type 1d they are more loosely packed. Additionally, a common crow was found infected with Types 1a, 1b, and 1d.

Type 2 cysts could readily be distinguished from each other on the basis of the proximity and lengths of the villi. Type 2a cysts had the spines very closely applied to each other and the endozoites densely packed (Plate 7), while Type 2b cysts had villi with readily visible spaces between them and endozoites loosely packed (Plate 8). That this is not an artifact is evident from the finding of both types in the same white-winged scoter. This does not preclude different ages for the cysts, but this is considered unlikely for the same reasons that cyst Types 1a to 1d are not believed to reflect age differences. Cyst Type 2c was readily distinguishable from the first two in this category by the length of its villi. Interestingly, birds infected with Type 2c cysts were not concurrently infected with cysts of another type.

Another difference between cysts of Type 2a and 2c is the degree of compaction of the endozoites within the two cyst types. If these two types represent developmental differences of the same parasite species, one would expect to find intermediate forms in a survey of this kind, but such intermediates were not found. Mehlhorn *et al.* (1975b) have shown that at least three species of parasite are involved in the *S. "fusiformis"* complex. Morphological criteria which allowed them to differentiate between the three species in the cystic stages include arrangement and appearance of the villi and the degree of compaction of the endozoites. Comparing their results with mine leads me to conclude that each of the types in the second category of cyst (Types 2a, 2b, 2c)

represents different species of *Sarcocystis*. Similarly, the first three in Type 1 series may also represent different species of avian *Sarcocystis*. I am less sure of Type 1d. Further investigation is needed to confirm whether Type 1d is a valid type. Thus, there may be six or more microcyst-producing species of *Sarcocystis* in birds. If the macrocyst-producing *S. "rileyi"* is added to this figure, there may be seven or more species of *Sarcocystis* in the avian host. At this time, it is sufficient to point out that several species may be present in the avian host. To attempt to discuss the taxonomy of this group is premature because neither are their life cycles known, nor has any of their developmental biology been described.

Life Cycle Experiments

Selection of the carnivore species to be used for the elucidation of the life cycle of the macrocyst-producing parasite of ducks was based on the ecological consideration of which carnivores were probable candidates (it was assumed that the parasite was transmitted in Alberta). Sargeant *et al.* (1973) demonstrated that ducks were vulnerable to predation by mink, and Bursey and Burt (1970) described hunting behaviour of bobcats on the periphery of bogs. Coyotes are common on the prairies where many ducks are produced every year. Hence these carnivores seemed to be likely candidates for completion of the life cycle. The failure to complete the life cycle of the parasite in coyotes and beagles (both fall and spring series) is considered reasonable evidence to eliminate canids from the life cycle of this parasite.

Failure to complete the life cycle in kittens of known history with fresh parasite sarcocysts cannot be attributed to experimental conditions. The results of the ferret experiments are less easily

interpreted because the kits were all infected with an unidentified *Isospora* upon their receipt. The immunological effect this may have had on the ferrets is not known. However, after the duck *Sarcocystis* experiments were concluded, the ferrets were successfully re-infected with the same *Isospora* they had upon their receipt. If there was little immune response to the original coccidial infection, the ferrets probably did not develop immunity to a different *Isospora*, i.e., *Sarcocystis*. There seems to be little immunological cross-reaction between *Sarcocystis* and other two-host isosporans. Doby and Beaucournu (1973) demonstrated that sera of carriers of *Isospora hominis* (one form of *S. "fusiformis"*) and antigen of *Toxoplasma gondii* did not cross-react in the indirect immunofluorescence test, and Fayer (1974) indicated that beagles infected with enteric stages of *S. "fusiformis"* can be easily re-infected at a later date. Although proof is lacking, it seems reasonable to conclude that failure to infect the ferrets was because they are not appropriate hosts rather than because they were immune to *Sarcocystis*. Similarly, it seems probable that mink are also not suitable hosts.

Reasonable precautions to ensure the coccidia-free status of the kittens reared for the spring series of experiments were taken. Thus it is concluded that failure to complete the *S. "rileyi"* life cycle in them is because cats are not involved in the life cycle, rather than because they might have developed an immunity. It seems possible, however, that felids are involved in the life cycle of a microcyst-producing species of *Sarcocystis* in non-migratory birds. Interpretation of the results from the feeding to one kitten of 8,000 oocysts (*I. felis?*) from the feces of another kitten previously fed infected black-billed magpie flesh is difficult. Had more oocysts been available for infecting additional

kittens with larger doses we could more confidently say that these oocysts were not infective for cats. However, Wallace (1973) was unable to initiate oocyst production in cats infected with 400,000 to 800,000 oocysts from cats fed a murine *Sarcocystis*. My experiments must be repeated, and the results duplicated before any conclusions can be reached.

Establishment of a sarcocyst infection in intermediate hosts with the oocysts from the black-billed magpie-cat cycle would have contributed considerably to the proof of the life cycle. Failure might be interpreted as an indication of a spurious *I. felis* infection in the kittens, and the oocysts were not infective to the birds. Frenkel and Dubey (1972), however, have shown that both *I. felis* and *I. rivolta* can use rodents as "intermediate" hosts. They (Frenkel and Dubey, 1972) fail to mention the presence or absence of cysts, but Wallace and Frenkel (1975) said distinct tissue cysts were reported in the former. Wallace (1973b) induced sarcocysts in mice after feeding them oocysts resembling those of *T. gondii*. In only one instance did an inoculum of less than 40,000 oocysts produce an infection in a mouse. The inocula used in my experiments were only 3,000 and 6,000 oocysts per bird. Use of large inocula, had the oocysts been available, may have successfully induced sarcosporidiosis.

There is evidence suggesting that the early series of experiments did not fail because of the time delay between death of the infected hosts and the feeding of the sarcocysts to the carnivores. Gestrich (1974) has shown that sarcocysts of one species of the *S. "fusiformis"* complex are infective up to 14 days after death of the host if the meat was kept refrigerated (but not frozen). Although the period in which avian

Sarcocystis remains viable after the death of the host is not known, Gestrich's results suggest that it may well exceed the 48 hours of two of the early experiments, and possibly the six days which occurred in Experiment No. 4.

Miller *et al.* (1972) showed that *T. gondii* can be passed to new intermediate hosts by feeding them cysts from chronically infected mice. Infection in new hosts was characterized by the reestablishment of the chronic stage (cysts) without the passage of oocysts. *Besnoitia* sp. (a parasite related to *Sarcocystis*) can be passed to new intermediate hosts in the same way (Frenkel, 1973). Both *T. gondii* and *Besnoitia* sp. can be transferred experimentally by injection of either endozoites from cysts, or by injection of rapidly dividing pre-cystic "tachyzoites". Unlike *T. gondii* and *Besnoitia* sp., *Sarcocystis* has not been transferred this way since the early reports of Darling (1910a) and Negri (1908). Similar attempts to transmit *Sarcocystis* failed in this study also.

Sarcocystis is known to be a two-host parasite in mammals (Rommel *et al.*, 1972; Heydorn and Rommel, 1972; Fayer and Johnson, 1974). How can the presence of cysts (indicative of the intermediate host) in the muscles of carnivorous birds be explained? Owls examined in this study and several early host-record reports for hawks (Darling, 1915; Osterud and Bascomb, 1928; Takos, 1957) show that they are suitable hosts for development of cystic stages of *Sarcocystis*, and that they are infected relatively frequently. Since ingestion of the cysts by a carnivore is required for completion of the life cycle, then, if the cystic stages in a given host are to complete the life cycle, they must be ingested by some carnivore. What preys upon birds of prey? Big hawks are known to prey

upon smaller hawks (Snyder and Snyder, 1974), but this does not seem to be a common occurrence. One explanation is that they are accidentally infected with the cystic stages of the parasite; it is a dead end with respect to the parasite's strategy for transmission. Another explanation is that the parasite uses scavengers to complete its life cycle, and depends upon the hosts being eaten by scavengers after the hosts' death.

An evaluation of the potential role of birds of prey as definitive hosts for *Sarcocystis* was not attempted because they were not available for use in feeding experiments. An attempt was made to investigate the potential role of avian scavengers in the epizootiology of *Sarcocystis*, but problems in rearing coccidia-free black-billed magpies made the results from this portion of the study unreliable. However, they can be considered as suggestive that these birds do not represent the "carnivore" part of the life cycle.

Whether the increased passage of *Isospora* sp. oocysts in the group of magpies fed *Sarcocystis* was due to the feeding of the sarcocysts or due to a higher rate of re-infection with the *Isospora* in this group is not known. There is a chance that a challenge with *Sarcocystis* might have provoked the increased output of *Isospora* oocysts. Campana-Rouget *et al.* (1974) reported that a *T. gondii* challenge of a kitten passing *I. felis* resulted in an increase of the output of oocysts of *I. felis* without producing an infection of *T. gondii*. Similarly, an *I. felis* challenge of a kitten infected with *T. gondii* resulted in a short-lived, but dramatic increase in the output of *T. gondii* oocysts and only a trickle of *I. felis* oocysts. An alternate (and more likely) explanation is that the birds in the test cage re-infected themselves more heavily through fecal contamination of their food than did those in the control group. Further

experimental research is needed to clarify my initial observations.

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Appendix 1. Common and scientific names of birds cited in the text.

Family Anatidae	
American wigeon	*Anas americana*
Blue-winged teal	*Anas discors*
Gadwall	*Anas strepera*
Green-winged teal	*Anas crecca*
Mallard	*Anas platyrhynchos*
Mottled duck	*Anas fulvigula*
Pintail	*Anas acuta*
Shoveler	*Anas clypeata*
Canvasback	*Aythya valisneria*
Lesser scaup	*Aythya affinis*
Redhead	*Aythya americana*
Bufflehead	*Bucephala albeola*
Common golden eye	*Bucephala clangula*
Ruddy duck	*Oxyura jamaicensis*
White-winged scoter	*Melanita deglandii*
Whistling swan	*Olor columbianus*
Family Gaviidae	
Common loon	*Gavia immer*
Family Podicipedidae	
Red-necked grebe	*Podiceps grisegena*
Western grebe	*Aechmophorus occidentalis*
Family Ardeidae	
American bittern	*Botaurus lentiginosus*
Great blue heron	*Ardea herodias*
Family Rallidae	
American coot	*Fulica americana*

Family Scolopacidae	
Common snipe	*Capella gallinago*
Greater yellowlegs	*Totanus melanoleucus*
Lesser yellowlegs	*Totanus flavipes*
Pectoral sandpiper	*Calidris melanotos*
Short-billed dowitcher	*Limnodromus griseus*
Willet	*Catoptrophorus semipalmatus*
Family Laridae	
Black tern	*Chlidonias niger*
Bonaparte's gull	*Larus philadelphia*
California gull	*Larus californicus*
Franklin's gull	*Larus pipixcan*
Ring-billed gull	*Larus delawarensis*
Family Phasianidae	
Ring-necked pheasant	*Phasianus colcicus*
Japanese quail	*Coturnix coturnix*
Family Tetraonidae	
Blue grouse	*Dendragopus obscurus*
Ruffed grouse	*Bonasa umbellus*
Sage grouse	*Centrocercus urophasianus*
Sharp-tailed grouse	*Pedioecetes phaseanellus*
Willow ptarmigan	*Lagopus lagopus*
Family Falconidae	
Cooper's hawk	*Accipiter cooperii*
Swainson's hawk	*Buteo swainsoni*

Appendix 1. (Continued)

Family Strigidae	
Boreal owl	*Aegolius funereus*
Great grey owl	*Strix nebulosa*
Great horned owl	*Bubo virginianus*
Snowy owl	*Nyctea scandiaca*
Family Corvidae	
Black-billed magpie	*Pica pica*
Common Crow	*Corvus brachyrhynchos*
Grey jay	*Perisoreus canadensis*
Family Turdidae	
American robin	*Turdus migratorius*
Family Bombycillidae	
Bohemian waxwing	*Bombycilla garrulus*
Family Sturnidae	
European starling	*Sturnus vulgaris*
Family Icteridae	
Brewer's blackbird	*Euphagus cyanocephalus*
Brown-headed blackbird	*Molothrus ater*
Common grackle	*Quiscalus quiscula*
Red-winged blackbird	*Agelaius phoeniceus*
Yellow-headed blackbird	*Xanthocephalus xanthocephalus*
Family Fringillidae	
Evening grosbeak	*Hesperiphona vespertina*
Hoary redpoll	*Acanthis hornemanni*
Snow bunting	*Plectrophenax nivalis*

Appendix 2. Common and scientific names of mammals cited in the text.

Domestic cattle (beef)	*Bos taurus*
Bobcat	*Lynx rufus*
Domestic cat	*Felis (?domesticus)*
Coyote	*Canis latrans*
Domestic dog	*Canis familiaris*
Ferret	*Mustela nigripes*
Laboratory rat	*Rattus sp.*
Mink	*Mustela vison*
Mule deer	*Odocoileus hemionus*
Pig	*Sus scrofa*
Domestic sheep	*Ovis aries*
White-tailed deer	*Odocoileus virginianus*

Appendix 3. Historical Overview.

Many researchers working with *Sarcocystis* begin their reports by saying that little is known about this cosmopolitan parasite. Although its existence was established by Miescher in 1843 and many researchers have worked with it since, it was with the elucidation of the life cycle of the closely related parasite, *Toxoplasma gondii*, that the clue to solving the life cycle of *Sarcocystis* was found. It is now known that *Sarcocystis* is a two host sporozoan having certain affinities with the coccidian genus *Isospora*. These are best demonstrated in enteric stages in a carnivorous "definitive host" while the cystic stages occur in other "herbivorous hosts".

The name *Sarcocystis* is descriptive in nature; it means literally "cysts (which occur as elongated whitish bodies) in the muscles". The cysts very often are microscopic in size, varying from a few micrometers to 100 or more micrometers in diameter, and 20 µm to several millimeters in length. Occasionally, in some animal species, cysts of two to three millimeters in diameter and up to 20 millimeters in length have been found. Cysts requiring histological techniques and the use of a microscope for their detection will hereafter be referred to as microcysts, while those readily visible to the unaided eye will be referred to as macrocysts. Collectively, they will be called sarcocysts.

The wall of the cyst is composed of a single, or double layered membrane (Weber, 1910; Crawley, 1911; Alexeieff; 1913; Chatton and Avel, 1923; Scott, 1943a; Ball, 1944; Ludvik, 1960; Zeve *et al.* 1966, Senaud, 1967) which may be smooth, or have external villosities sometimes known as rods, prisms, cytophaneres, or radial spines (Weber, 1910; Chatton and Avel, 1923; Ludvik, 1959; Senaud, 1967). The primary wall which is present

in all sarcocysts is thin, may give rise to villosities on the outside, and thin septa on the inside dividing the cyst into compartments (Senaud, 1967). The internal septa are not limited by a unit membrane (Senaud, 1967; Scholtyseck *et al.*, 1974).

The morphology of the cyst wall of different sarcocysts has been described (Bertram, 1892; Laveran and Mesnil, 1899; Ferret, 1903; Weber, 1909, 1910; Alexeieff, 1913; Chatton and Avel, 1923). Most reported seeing villosities, but there was some disagreement among them as to their nature; were they part of the cyst itself or artifacts (see Alexeieff, 1913). Precise descriptions of the cyst wall were virtually nonexistent until Ludvik (1960) began ultrastructural studies of *S. miescheriana* from pigs. Zeve *et al.* (1966), in an ultrastructural study of cysts of *Sarcocystis* sp. from grackles mentioned the presence of villosities continuous with the cyst wall, but neglected to give any idea of size, length, or number. Simpson and Forrester (1973) added little to our knowledge of the cyst wall. To my knowledge there is no other recent study of the cyst wall from the avian host. Since adequate study of the development of the cyst wall in any given host depends upon being able to experimentally infect that host, it is not surprising that the first developmental studies did not begin to appear before those of Mehlhorn *et al.* (1975a, b), Heydorn *et al.* (1975a, b), and Gestrich *et al.* (1975). On the basis of life cycle, biology, and of developmental morphology in the intermediate host they have been able to show that bovine *S. "fusiformis"* is a composite of no less than three distinct species. The only comparative study of cysts to date is that of Kaliner (1975) who reported finding different cyst types in a number of different African game mammals. He reported different cyst types in the same individual, and occasionally in

the same muscle cell, but did not discuss the significance of his findings.

The presence of the outer, or secondary, cyst wall is variable, apparently depending in part upon the age and size of the cyst and in part upon the species of parasite (and/or host). In macrocysts of *S. "tenella"* from sheep the secondary wall consists of the remnants of the infected muscle fiber, and hypertrophied connective tissue which once bounded it (Alexeieff, 1913, Senaud, 1967). The secondary wall constitutes part of a host response to the parasite, is not a part of the cyst proper, and never supports cyst-related structures such as septa or villosities.

The role of the host cell and of the parasite in the construction of the primary wall is not yet well understood. Many workers considered the primary wall to be of parasite origin, but it has recently been shown to develop from a parasitophorous vacuole wall laid down by the host cell (Mehlhorn *et al.* 1975a). It is only after the parasite has begun division that the vacuole becomes elaborated into the more complex wall usually seen.

Many cells which have been called "spores", "Rainey's bodies", "sporozoites", "schizozoites", "merozoites", "cystozoites", "endodyocytes", "endozoites", "trophozoites", and "bradyzoites" are found in the interior of the cyst. Many workers use the term merozoite because these cyst zoites morphologically are very similar to merozoites of other coccidia (Senaud, 1963). They develop inside the cyst from a mother cell, sometimes referred to as "cytomere" or "sporoblast", but now called "metrocyte" (Senaud, 1967). Metrocytes undergo a special kind of binary fission known as endodyogeny, first observed by Goldman *et al.* (1958) rather than schizogony, a form of multiple fission by which merozoites in many typical coccidians are formed.

There is still some controversy over the nature of endodyogeny, that is, is it fundamentally different from schizogony, or is it just a simplified version of schizogony (see Aikawa and Sterling, 1974)? Until such time as the homologies between merozoites from enteric stages of coccidian life cycles and "zoites" from their cystic stages are better understood, it may be advisable to use a different term. Frenkel (1973) has proposed the term "bradyzoite" for these cells, because, he says, other terms are vague and lack precision. In the first good description of the process of endodyogeny, Senaud (1967) used the term endodyocyte for the products of this kind of cell division; thus endodyocyte has priority over Frenkel's bradyzoite, but is a difficult word to articulate. If a special term other than merozoite is to be used, endozoite is better than bradyzoite because it is descriptive and avoids introducing yet another specialized term into the vocabulary.

The widespread occurrence of *Sarcocystis* in some animals made it the subject of intensive investigations, including many attempts to solve the enigma of its life cycle. Smith (1901, 1905) reported inducing sarcosporidiosis in uninfected laboratory mice by feeding them carcasses of mice infected with *S. muris*, with a delay of about five months between the feeding of infected meat and the appearance of cysts. Negre (1907, 1910) infected healthy mice with *S. muris* by feeding them either sarcocysts or fecal material from mice previously fed cysts of *S. muris*. The prepatent period was six weeks, with about 90 days required for the appearance of mature cysts. In light of recent discoveries, it is interesting to note that Negre reported in his mice the presence of intestinal stages which had the ability to infect healthy mice. His description of parasites in the intestinal cells can be interpreted as develop-

ing oocysts. Negre was unable to transmit *Sarcocystis* either intraperitoneally or subcutaneously. Darling (1910b, 1915) reported transmitting *Sarcocystis* from rats to guinea pigs by injecting endozoites intramuscularly, but his description of the ensuing infections suggests a concurrent infection with *Toxoplasma*. Scott (1915) was unable to infect lambs by feeding them cysts from sheep. In an experiment which could have resulted in the discovery of the life cycle of *S. "tenella"* (and possibly provided the clue to the life cycles of other sarcosporidians) Scott (1915) fed infected sheep flesh to dogs and then allowed them to run about freely in an enclosed pasture where lambs were subsequently enclosed. Unfortunately, the dogs were removed 14 days after the first feeding of infected meat. Had he waited longer, or had he used cats, the transmission might have been successful. Scott (1943a) presented a review of the literature, and concluded that *Sarcocystis* had a direct life cycle. He postulated that the parasite's spores (endozoites) left the muscle cysts and migrated to the intestine by an unknown mechanism, passed out with the feces, and infected other animals that came in contact with the feces.

More recently Spindler *et al.* (1946) showed that a fecal stage was required in the life cycle of *S. miescheriana* of pigs. They fed cysts to a variety of animals including cats, dogs, rats, pigs, and chickens. After fifteen days the feces of these animals induced sarcocysts in the muscles of uninfected pigs. Pigs fed cysts did not in turn develop cysts if copraphagy was prevented. Pursuit of this clue would probably have resulted in the discovery of the life cycle at that time.

Unfortunately, Spindler *et al.*'s work went largely unnoticed, and it was only with the elucidation of the life cycle of *Toxoplasma*

gondii by Hutchison (1965, 1967) and Work and Hutchison (1969) twenty years later that renewed interest was generated in the question of the sarcosporidian life cycle. Fayer (1970) reported the transformation of endozoites of a sarcosporidian from grackles into gametocytes and oocysts in cell culture. Rommel *et al.* (1972) demonstrated isosporan oocysts and sporocysts in the feces of cats fed sheep flesh infected with *S. "tenella"*. Sarcocysts of *S. "fusiformis"* were induced to develop in dogs and cats fed infected beef (Heydorn and Rommel, 1972). During the first days of the patent period, two sporulated sporocysts were frequently seen envelloped by a thin sheath, giving them an isosporan appearance. Later in the patent period, however, the sporocysts were not usually associated with a sheath. The *S. "fusiformis"* life cycle was repeated using beagle pups (Mahrt, 1973) and sarcosporidiosis was then induced in calves by feeding them sporocysts from the beagles (Fayer *et al.* 1973; Fayer and Johnson, 1974). As a result of this information it is now known that these parasites infect new hosts by the oral route, via the ingestion of oocysts or sporocysts in the case of intermediate hosts, and via the ingestion of cyst-infected meat in the case of carnivores.

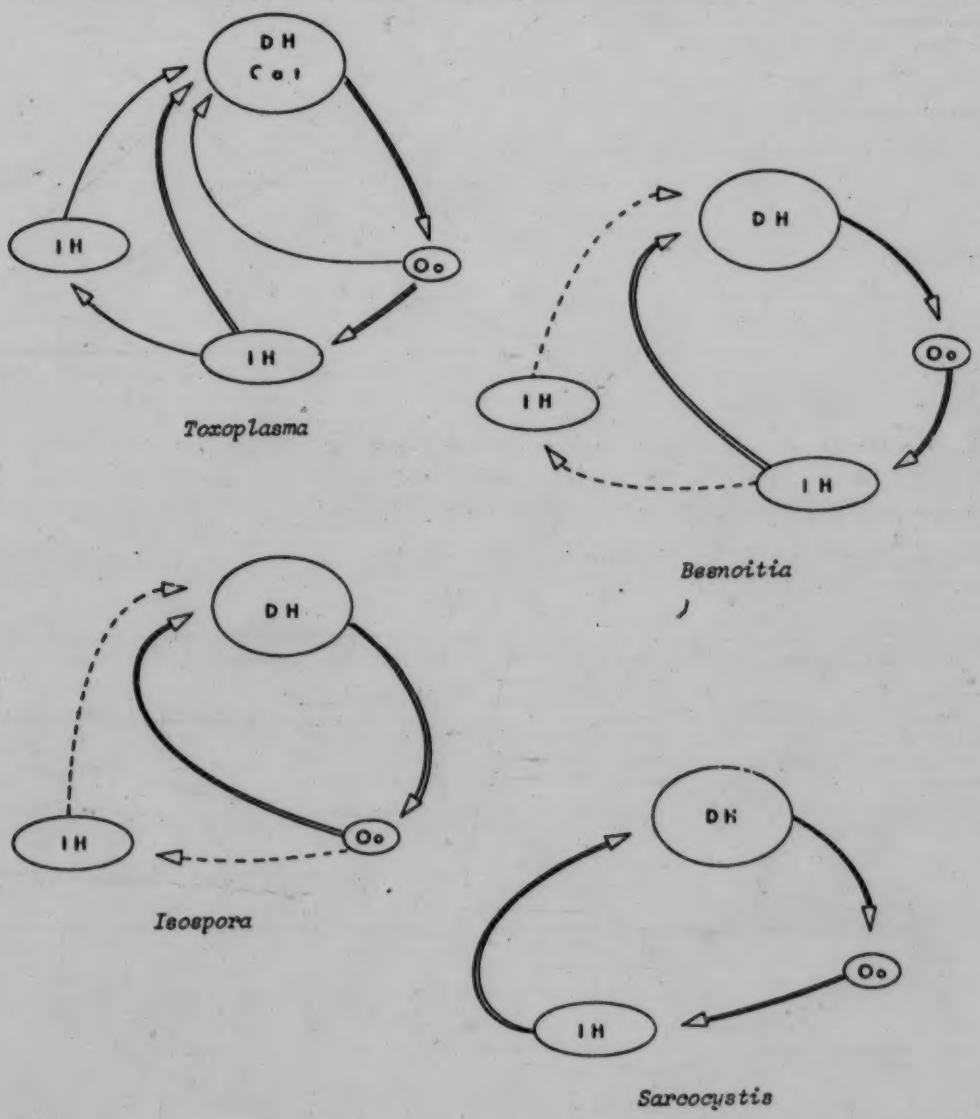
In earlier days, and to some extent more recently, separation of *Sarcocystis* into species was based on the host species infected, morphology (both gross and micro) of the cyst, and size of the enclosed zoites. Genera were defined on the basis of the specific tissue infected; *Sarcocystis* was found exclusively in muscle fibres, *Besnoitia* developed only in connective tissue, *Toxoplasma* had little site specificity, and *Frenkelia* was found exclusively in the brain. Recently, *Hammondia* n. gen. which also forms intramuscular cysts was added to the group (Frenkel and Dubey, 1975a, b). Recent studies, especially those by Fayer *et al.*

(1973), Fayer and Johnson (1973, 1974), and Johnson *et al.* (1975) have shown that this site specificity in the host, especially in pre-cystic stages, is not as rigid as was previously thought.

Despite some overall similarities in the biology of these organisms, there are noticeable differences. Only in *Toxoplasma* are oocysts infective to both intermediate and definitive hosts (i.e., cats); in all other parasites where life cycles are known, oocysts or sporocysts are infective only to intermediate hosts. In *Toxoplasma*, and in one species of *Besnoitia*, tachyzoites (rapidly multiplying stages present in macrophages; this is a precystic stage) and the endozoites are directly infective either by subinjection, or oral ingestion, to other intermediate hosts (Wallace and Frenkel, 1975). Such is not the case with either *Sarcocystis* or *Hammondia*. The similarities and differences in the life cycles of the various groups are depicted in Figure 2.

In addition, available information indicates that only in some (*Toxoplasma, Besnoitia,* and *Hammondia)* does schizogony preceed gametogony in the intestinal epithelium of the definitive host (Frenkel and Dubey, 1975a, b: Wallace and Frenkel, 1975). Species of *Sarcocystis* are not known to have a schizogonic stage preceding gametogony (Fayer, 1974). In those parasites with the schizogonic cycle, oocysts are passed for relatively short periods (i.e., 4 to 20 days) while in the case of *Sarcocystis ("fusiformis"* and *"tenella")* the patent period is from 20 to 70 days(Rommel *et al.* 1972; Heydorn and Rommel, 1972; Fayer, 1974). If schizogony does not occur in the gut of the definitive host and oocysts or sporocysts are passed for up to 70 days, it is reasonable to suspect that schizogony is occurring elsewhere in the host's tissues. Fayer (1974) did not report if these stages were looked for in the tissues of

Figure 2. Schematic representations of the known life cycle pathways of the cyst-producing coccidia.



D H = Definitive host
O o = Oocysts or sporocysts
I H = Intermediate host

Broken line indicates pathways some members in the group are known to use, but are not used by other species in the group.

Double lines indicate the usual life cycle route taken by members of the group.

Single line indicates alternate pathways available to the parasites.

dogs (other than in the gut) with patent infections.

Elucidation of the life cycle of *Toxoplasma gondii* has demonstrated that one stage of its life cycle is indistinguishable from one of the two forms of *Isospora bigemina* (small form). Wallace (1973, 1975) found two sarcosporidians in mice, one of which could be induced in mice by feeding them oocysts indistinguishable from those of *T. gondii*, but which was biologically distinct from *T. gondii*. This parasite is referred to as "WC 1170" by Wallace. The causative agent of the second sarcosporidian was not identified. Tadros *et al.* (1974) showed that sera from carriers of *I. hominis* and antigen of one form of *S. "fusiformis"* cross-react. Feeding infection-free calves with oocysts of *I. hominis* (obtained from human volunteers fed *Sarcocystis*-infected beef) resulted in a characteristic kind of sarcocyst in the calves (Mehlhorn *et al.*, 1975a). Feeding *S. "tenella"* to cats resulted in the passing of sporulated isosporan sporocysts (Rommel *et al.*, 1972). Parasites of the *S. "fusiformis"* complex, when fed to cats, produced isosporan sporocysts (Heydorn and Rommel, 1972), when fed to dogs produced isosporan sporocysts (Rommel and Heydorn, 1972; Mahrt, 1973; Fayer *et al.*, 1973; Fayer and Johnson, 1974); and when fed to man produced oocysts of *I. hominis* (Mehlhorn *et al.*, 1975a). Oocysts of *I. felis* taken from cats and fed to rodents produce simple cysts in the tissues of the rodents which in turn are infective to other cats (Frenkel and Dubey, 1972). And cysts of *Besnoitia* sp., when fed to cats, produce *Isospora*-like oocysts (Wallace and Frenkel, 1975). Clearly, it is impossible to differentiate between the oocysts of many "classical" *Isospora* and the oocysts (and/or sporocysts) produced by the various cyst-forming parasites.

Since the recent discoveries of the life cycle of *Sarcocystis*, two rather different opinions regarding the classification of the group have developed. On the one hand is J. K. Frenkel who maintains that there are sufficient differences between the parasite groups to separate them into three families, i.e., the Eimeriidae (in which he includes *Eimeria* and *Isospora* "*sensu strictu*"), Sarcocystidae (in which he includes *Sarcocystis*, *Frenkelia*, and *Hammondia*), and Toxoplasmidae (in which he includes *Toxoplasma* and *Besnoitia*) (Frenkel, 1974). On the other hand the Europeans (including Scholtyseck, Senaud, Mehlhorn, Heydorn, Rommel, and others) suggest that the cyst-forming coccidia probably all belong with the genus *Isospora*.

In his proposed taxonomic scheme, Frenkel (1974) gave major importance to the variations in the life cycles of the different parasites and the degree of sporulation of the oocysts (and/or sporocysts). Thus those typified by a one-host life cycle (monoxenous) are placed in a single family, the Eimeriidae, which includes *Eimeria*, *Isospora*, and *Atoxoplasma*. Those parasites having a two-host life cycle (heteroxenous), whether facultatively or obligatorily, are placed in either the Sarcocystidae (including *Sarcocystis*, *Hammondia*, and *Frenkelia*), or the Toxoplasmidae (including *Toxoplasma* and *Besnoitia*). Little attention was paid to the basic structure of the oocysts themselves, or to the ultrastructural similarities between the endozoites (Scholtyseck *et al.*, 1973).

Examination of the available information on these groups indicates certain weaknesses in the basis of this scheme. For example, can the parasites in Frenkel's Toxoplasmidae be separated from those in his Sarcocystidae? In Frenkel (1974), Table 5, p. 147, the first differ-

ence between these two families lies in the mechanism of cyst formation. In his toxoplasmids, the cyst develops "from a parasitophorous vacuole" while in the sarcocystids it develops "in and between host cells"!! This is clearly vague and insufficient. Moreover, it is inaccurate since it has been demonstrated (Mehlhorn *et al.*, 1975a) that cysts of *S. "fusiformis"* in calves begin as single-celled parasites (metrocytes) inside typical parasitophorous vacuoles formed intra-muscularly. The cyst wall is developed by progressive thickening and modification of the vacuole wall while the metrocytes within are dividing. Senaud (1969) demonstrated that the origin of the cyst wall in *Besnoitia jellisoni* (included by Frenkel in his Toxoplasmidae) is from an intramuscular parasitophorous vacuole laid down by the parasitized host cell, and that the vacuole membrane is anchored by villosities in a peripheral layer of collagen on the surface of the cyst wall.

It can be argued that the similarities in cyst wall development in Frenkel's Toxoplasmidae and Sarcocystidae, and development of the parasitophorous vacuole in endothelial cells by some *Eimeria* suggest a close affinity between these groups. For example, 2nd generation schizonts of *E. necatrix* caused cellular and nuclear hypertrophy and induced rounds of DNA synthesis in the host cell (Fernando *et al.*, 1974). Cells parasitized by *E. auburnensis* formed a parasitophorous vacuole with numerous folds of the cell membrane penetrating the vacuole (Hammond *et al.*, 1967). It was hypothesized that these folds helped in the transfer of nutrients to the parasites. Thus separating these parasites into separate families on the basis of the origin of the cyst wall seems unwarranted.

Other differences between the Toxoplasmidae and the Sarcocystidae

given by Frenkel cannot at this time be given serious taxonomic consideration beyond the species level. Selection of location for development of the cystic stages may depend on little more than the "niche" required by the parasite. Morphological differences between cyst walls are to be expected in different species, especially since there is evidence (Fernando *et al.* 1974) Hammond *et al.* 1967) that the parasite can "take over" and direct the host cell's metabolic functions. All parasites (as Frenkel admits) are intracellular, initially at least. Shape of endozoite (Frenkel's bradyzoite) i.e., broad vs. slender, is highly subjective. Passing unsporulated or sporulated oocysts, or sporulated sporocysts which are morphologically similar have been shown to be due to differences in developmental rates (Mehlhorn and Scholtyseck, 1974).

A major difficulty which Frenkel did not resolve was what to make of the similarities and differences in the structure of the oocysts. The oocysts of *Eimeria* have four sporocysts, each containing two sporozoites (Levine, 1973). The oocysts of *Isospora* have two sporocysts, each containing four sporozoites. Both, though morphologically different, have previously been placed in the family Eimeriidae (Scholtyseck, 1974), where Frenkel leaves the *Eimeria* and some (only) of the *Isospora*. He did not resolve the difficulty of justifying the separation of some *Isospora* into one family and combining other *Isospora* with the *Eimeria* in a different family. The problem is that, where life cycles are presently known, each of Frenkel's toxoplasmids and sarcocystids pass oocysts or sporocysts indistinguishable from those of the "classical" *Isospora*. In addition it has been shown (Frenkel and Dubey, 1972) that some "classical" *Isospora*, e.g., *I. felis* and *I. rivolta*

(coccidia which Frenkel left in his Eimeriidae) can facultatively use intermediate or transport hosts with the production of muscular cysts!

Hammondia hammondi n. gen., n. sp. (in honor of the late D. M. Hammond) was created for a sarcosporidian isolated from the feces of a stray cat (Frenkel and Dubey, 1975a, b). Production of skeletal muscle cysts is a "pronounced" feature of the new genus, but it differs from *Sarcocystis*

> "by slender bradyzoites [endozoites] , thin-walled cysts, absent radial spines, septa, or metrocytes, by a multiplicative cycle in the gut of the final host (which is lacking in all *Sarcocystis* so far studied), and by shedding of unsporulated oocysts (whereas all known *Sarcocystis* shed sporulated sporocysts or oocysts)."

Later they say it (*Hammondia*) differs from the "common *Isospora*" but fail to point out the differences (Frenkel and Dubey, 1975b, p.224).

The validity of the structure of the cyst walls, the shape of the endozoites, and the shedding of oocysts as taxonomic criteria above the species level has been discussed already. Since they did not report having done extensive developmental work on the cysts, and did not report having done any electron microscopy, the absence of metrocytes in the cysts may be due to their failure to recognize them. Thus creation of a new genus on the basis of the criteria reported by Frenkel and Dubey (1975a, b) appears premature at this time, and unfortunately the taxon *Hammondia* in now preoccupied, and may well become a nomen nudum.

Whether or not *Sarcocystis* causes pathological problems in the intermediate host has long been a controversial question. Failure to find any tissue reaction such as inflammation around the cysts, and the obvious good health of many infected hosts led some workers to conclude that the presence of the parasite was of little consequence to its

host. On the other hand, considerable evidence has accumulated to indicate that it is not always innocuous. Thus, an aqueous extract of *S. "tenella"* sarcocysts from sheep when injected into rabbits caused their rapid death (Pfeifer, 1891). Laveran and Mesnil (1899) confirmed Pfeifer's earlier report and then described some of the symptoms preceding death of the rabbits but were unable to induce similar reactions in rats, mice, sheep, chickens, pigeons, frogs, and turtles. A strong generalized leucocytic reaction preceding the formation of muscle cysts in mice infected orally with *S. muris* was described by Negre (1907). Equine sarcocysts had the same effect on rabbits as did sheep sarcocysts (Sabrazes and Muratet, 1911). Besnoit and Robin (1913) described an intense leucocytic infiltration in the vicinity of bovid "cutaneous" sarcocysts which, they suggested, resulted in the breakdown of the cysts. Serious muscle degeneration accompanied by a serous accumulation in muscles of pigs was reported by Creech (1922). Spindler *et al.* (1946) found that hogs experimentally infected with porcine *S. miescheriana* experienced vomiting, inappetance, pale enlarged kidneys, retarded growth rate, and muscular weakness in the loins. Fayer *et al.* (1973) reported serious illness and death in calves fed *S. "fusiformis"* sporocysts from dogs.

Although it is generally agreed that there is little or no histopathology visible in the vicinity of intact sarcocysts, and that most infected animals appear normal, the toxic reactions reported by Pfeifer, Laveran and Mesnil, and Senaud; the histopathology described by Negre, Besnoit and Robin, Fayer *et al.* and Senaud; and the clinical symptoms described by Spindler *et al.* and Fayer *et al.* are all indicative of pathology. Scott (1943b) presented a comprehensive review of the

literature to that time which left little doubt that *Sarcocystis* is in fact pathogenic under certain conditions.